STUDIES OF THE IMMUNE RESPONSE OF LABORATORY MICE TO SEQUENTIAL FEEDINGS OF TWO SPECIES OF ARGASID TICK

By

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TO LOGAN, KYLE, and SHARON,

FOR YOUR LOVE, JOY, and FAITHFULNESS.

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STUDIES OF THE IMMUNE RESPONSE OF LABORATORY MICE TO SEQUENTIAL FEEDINGS OF TWO SPECIES OF ARGASID TICK

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A study was conducted to examine the host immune responses of laboratory mice to sequential feedings of two species of Ornithodoros tick. One species, O. turicata, feeds in less than one hour in all instars, typical of many argasid species. The other species, O. talaje, attaches for six to eight days during its larval feeding stage, but feeds similarly to O. turicata as a nymph and adult.

Acquired host resistance developed in laboratory mice after sequential exposures to the above-mentioned species. Host resistance manifested itself in reduced weight gains of O. turicata larvae during the second and third exposure times. Mortality of O. talaje increased significantly upon second and third exposure to the same host compared to that seen upon naive mice. In addition, while attachment time of O. talaje larvae increased, detachment weight did not.

Significant antibody responses were detectable for at least 90 days after last exposure in mice as a result of sequential larval argasid tick exposures. Antibody isotyping showed that the initial response was of the IgM class with a secondary class switch to the IgG1 subclass. Cross-reactive salivary gland proteins were found in argasid and ixodid ticks.

Several different cross-reactive proteins were demonstrated by Western blotting in both argasid and ixodid tick salivary glands as well as in a Psoroptes mite, though no proteins were common to both the tick families and the mite. One protein was cross-reactive between the two tick families.

Histological and in vitro studies showed that a cell-mediated host response occurred resulting from sequential exposures to O. talaje larvae. A definite shift during first to third exposure from neutrophils to lymphocytes, probably T cells, was seen at both the attachment site and inside the tick.

Immunization of mice with various crude extracts of tick parts induced high host antibody levels that could be detected in the hemolymph of recently fed ticks. In the case of O. turicata, it had no effect on tick survival. In the case of O. talaje, it induced a decrease in survival comparable to that of naturally acquired resistance.

CHAPTER 1
AN INTRODUCTION TO THE SUPERFAMILY IXODOIDEA AND A DISCUSSION OF HOST IMMUNE RESPONSE MECHANISMS TO TICK FEEDING

Classification of the Ixodoidea

Morphology

Ticks are in the phylum Arthropoda, due to their jointed legs and chitinous exoskeleton. Considerable differences exist that clearly delineate them from others in that phylum. As members of the subphylum Chelicerata, their primary mouth parts are the chelicerae rather than the mandibles of the insects. Ticks and mites are in the class Arachnida with scorpions, spiders, and harvestmen, but constitute the subclass Acari due to the fusion of major body parts and the apparent lack of abdominal segmentation (Teel, 1985).

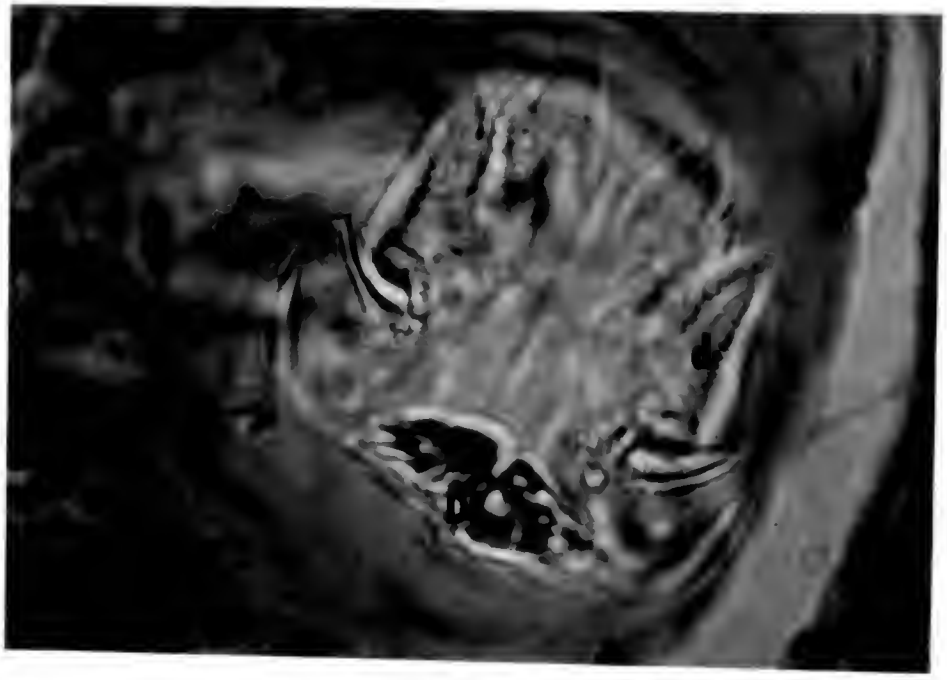
Ticks are further classified in the order Parasitiformes or Metastigmata, depending on the classification scheme used (Krantz, 1978), and in the superfamily Ixodoidea. Common to all ticks is the body plan consisting of two main regions: the anterior mouthparts (capitulum or gnathosoma) and the general body region that bears the legs and unsegmented abdomen (idiosoma).

The chelicerae and palps are located on the capitulum. The dorsal chelicerae are two-segmented appendages housed in cheliceral sheaths and are capable of anterior-posterior motion. The distal end of each chelicera has a sclerotized, toothlike digit which moves laterally to cut the host's skin during attachment (see Figure 1.1). The hypostome, located beneath the chelicerae, acts as the floor of the oral cavity, and its ventral surface is armed with recurved denticles which facilitate attachment to the host. The idiosoma bears six legs in the larva and eight legs in the nymph and adult, which are all capable of considerable articulation. Located on the dorsal surface of tarsus I is the opening to a cavity known as Haller's organ, in which are situated numerous chemoreceptor-type setae involved in the feeding process (Teel, 1985).

The Ixodoidea include all three families of ticks: Argasidae, Ixodidae and Nutalliellidae. The latter family is represented by a single rare African species and will not be considered further. More than 800 species of the other two families have been described from throughout the temperate and tropical regions of the world, all of which feed obligately on the blood of mammals, reptiles, or birds (Doss et al., 1974). The main difference in appearance of the two families is in their dorsal aspect. The Ixodidae have a hard dorsal shield or scutum which is sometimes characteristically marked, covering the entire back of the male and a portion of the female. Their mouthparts are

Figure 1.1. A. Chelicerae of attached Onithodoros talaje larva showing sclerotized cutting surfaces of the digits as well as the hypostomal denticles.
B. SEM picture of the mouthparts of O. talaje larva for comparison, (660x).
(D) Digits, (H) Hypostome, (d) denticles, (P) Palps.

A



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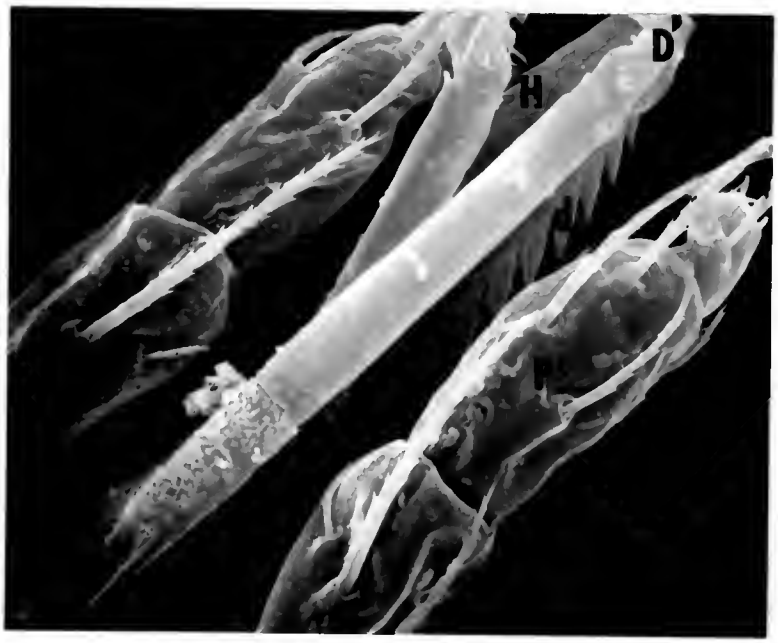

clearly visible at the anterior end of the body when observed from above. In the Argasidae, the scutum is entirely absent and the outer skin is a tough, leathery coat of uniform appearance all over the body. The mouthparts of the nymphs and adults are located ventrally and are not visible when observed from above (Lancaster and Meisch, 1986).

General Biology

The tick life cycle includes four stages: egg, six-legged larva, eight-legged nymph and adult. The Argasidae can produce from two to seven nymphal instars, depending on species, host, and environmental conditions. The Ixodidae produce only one nymphal instar.

Tick feeding begins with an incision in the host's skin made with the chelicerae. As the incision is made, the hypostome is inserted and the palps are spread on the surface of the host. The palps play a sensory role while the hypostome is used to anchor the tick during feeding. In certain ixodid species, salivary glands are known to secrete a cement-like substance to aid in attachment. In both families, anticoagulants as well as other pharmacologically active substances that play a role in feeding success are also secreted. As will be discussed later in greater detail, these same secretions are the causal factors for stimulating the host immunological response.

In the Ixodidae, the salivary glands provide the primary route of water regulation during feeding (Sauer, 1977). As the blood meal is concentrated in the gut, excess water and ions are passed to the hemolymph and eliminated by the salivary glands back into the host. In the Argasidae, the coxal glands (between coxae I and II) eliminate excess fluid from the hemolymph directly onto the host skin. There are a few species of argasid tick in which the larval stage must remain attached for several days to complete feeding. This prolonged attachment time is probably related to specialized physiological differences seen in at least Argas persicus (Oken), where coxal glands are not present until the nymphal stages (Moorehouse, 1975) and excess water and ions must be secreted slowly through the salivary glands. These salivary and coxal fluid excretions are the principal means for disease transmission (Teel, 1985).

Developmental and Behavioral Characteristics

In addition to the physiological and morphological differences that exist between the Ixodidae and the Argasidae, certain other basic differences should be noted (see Table 1.1). These differing characteristics have far-reaching impact when considering methods for tick control as well as when evaluating the epidemiology of tick-borne diseases.

Ixodid ticks are active hunters when searching for a host meal, feeding only once per life stage for a maximum of three host interactions. They usually complete their life cycle in less than 1.5 years. While feeding, ixodid ticks remain attached to their host for a minimum of several days, sometimes as long as 14 days. Mating occurs during or immediately after engorgement as adults, and soon thereafter a single batch of up to several thousand eggs is laid. As host interactions are limited to three feedings and the ixodids actively search for and attach to a host, tick control, though difficult, can be achieved. Disease parameters such as reservoir and alternate hosts can be effectively studied and understood.

Argasid ticks do not actively search for a host meal; rather, they remain in their host's habitat, usually a burrow or nest of some type, and feed upon animals entering that environment. Feeding is extremely fast, seldom taking more than one hour. Perhaps because of their nidiculous nature, they may feed several times in any one life stage, and frequently feed several times as adults. Mating occurs after feeding, and several separate, small batches of eggs may be laid resulting from a single blood-meal and mating. Argasid ticks live for several years and may survive much of that time under near-starvation conditions. As potential host interactions are unlimited, nonspecific, and of a very rapid nature, and the ticks are remote and very long lived, understanding the parameters of argasid

tick-borne diseases is very difficult (Balashov, 1968; Harwood and James, 1979).

Table 1-1. Developmental and behavioral differences between ixodid and argasid ticks.

Behavioral Characteristic	Ixodid	Argasid
Host finding strategy	active,hunter	fortuitous, nidiculous
Number of hosts	1-3	4 or more
Number of feedings-per life stage	1	>1
Number of feedings-as adult	1	>1
Length of feeding	5-10 days	30-120 minutes
Number of eggs laid/ Number of egg batches	1000s/one batch	100s/ >1batch
Sexual dimorphism	Yes	No
Longevity	1-1.5 years	>2, up to 19 years

Medical and Veterinary Importance

From a health perspective, ticks are an extremely important group of arthropods affecting nearly every terrestrial vertebrate, particularly mammals. Tick feeding causes host stress and blood loss, and even without the transmission of pathogens, causes significant harm to wild vertebrates as well as man's livestock and poultry. Losses in beef cattle due to ticks have been estimated at more than 25 million dollars annually in Australia. As long ago as 1965, the USDA estimated in the United States

annual tick-associated losses in cattle at 60 million dollars and at 4.7 million dollars in the sheep industry (Harwood and James, 1979). In 1982, it was estimated that on a world-wide basis, ticks affect 800 million cattle and an equal number of sheep (Sutherst et al., 1982).

Many disorders of man and animals are caused by ticks such as dermatosis, exsanguination, otoacariasis, as well as numerous infections including piroplasms, rickettsia, viruses, spirochetes, bacteria and filaria. In fact, ticks transmit a greater number and variety of diseases to domestic animals than any other arthropod, and are second only to mosquitoes as vectors of human disease (Obenchain and Galun, 1982). Several extensive reviews of tick-associated diseases have been completed, (Balashov, 1968; Doss et al., 1974; Hoogstraal, 1966, 1967, 1973, 1979, and 1985) though no recent work encompassing all diseases presently exists.

Various factors account for the effectiveness of ticks in the spread of human and animal diseases. Among these factors are (1) ticks are persistent bloodsuckers, (2) ixodid ticks are slow feeding, permitting ample time for pathogen transfer as well as dispersion of the individual ticks, (3) they utilize a wide host range, again insuring greater opportunity to acquire and transmit pathogens, (4) they have a relatively long lifespan, and (5) transovarial-transtadial transmission of some pathogens insures infectivity in the next generation (Harwood and

James, 1979). As the pressures of increasing human population cause man to move into areas once considered wild, the frequency and incidence of tick-borne disease will certainly increase (Hoogstraal, 1981).

Evolutionary History

Host-Tick Evolution

It is generally believed that parasitic arthropods such as ticks developed through a series of transitional stages from free-living to the parasitic stage. Initially, they were probably scavengers feeding on organic debris such as wastes and host remains within the lairs, burrows or nests of the host. Once that relationship was established it is easy to understand how closer relationships might have developed; keratinophagy to mucophagy or sarcophagy, leading eventually to hematophagy (Kim, 1985).

The Ixodoidea are thought to have first evolved as obligate parasites of the Reptilia in the warm, humid climate of the late Paleozoic era, approximately 250 million years ago (Hoogstraal, 1985). The radiations of the Reptilia during that timeframe stimulated the evolutionary radiation that divided the Ixodoidea into two distinct lines, Argasidae and Ixodidae. The argasid ticks developed a tendency towards feeding quickly and more frequently, remaining in the roost or nest area, while the ixodid ticks developed a tendency toward taking more time

to obtain a larger meal on a less frequent basis. Regardless of feeding strategy, it is believed that at that time both families of ticks fed regularly on blood at all stages of the lifecycle (Hocking, 1971).

At about the beginning of the Tertiary period, approximately 70 million years ago, primitive bird and mammal lines replaced reptiles as the dominant terrestrial vertebrates, and explosive radiation in the surviving tick lines paralleled that of the new vertebrates. Much coadaptation has occurred in the ensuing timeframe, resulting in a high degree of host specificity. For instance, in modern ticks, one finds a number of physical adaptations that appear common to most parasitic arthropods: dorsoventral flattening, with head and other structures modified accordingly (Kim, 1985). Specific morphological adaptations are seen in the attachment and feeding organs. In the Ixodidae, the long-toothed hypostome is sunk deeply into the skin, and attachment is aided by the cheliceral digits with recurved teeth. Also seen in the ixodid ticks is the cement produced by the tick during the early stages of feeding to assist in maintaining attachment (Binnington and Kemp, 1980). In the Argasidae, the hypostome is more delicate, not adapted for long periods of attachment, and the cheliceral digits are heavier and adapted for cutting host skin more rapidly (Balashov, 1968).

Host-Parasite Interactions

Mammals have been available as potential hosts for approximately 200 million years. Although little is known about the early interaction between parasites and their mammalian hosts, it is well documented that parasites, together with their hosts, underwent a tremendous evolutionary radiation. Mammals evolved to occupy a wide range of niches on land and in water at the same time arthropods were evolving to fill a wide array of niches available on the mammalian body (Timm and Clauson, 1985). This process of concurrent evolution is sometimes referred to as coevolution. As defined classically, such a process would involve "an evolutionary change in a trait of the individual of one population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change in the first" (Jantzen, 1980). In parasite-host systems, this degree of interaction is difficult to demonstrate, therefore a term such as co-accommodation may be more appropriate (Brooks, 1979).

Regardless of the exact terminology, the host is not simply a passive black box that provides a habitat, food, and other biological necessities for the parasite (May, 1983). In the host-parasite system, parasites exploit the host, and, in response, the host reacts to minimize that exploitation. The existing relationships between parasitic arthropods and mammals are a result of those interactions

over a long time (Kim, 1985). The central aspect of parasite associations is nutrient transfer and energy exchange (Wakelin, 1984). As parasites attach and feed they also introduce their own salivary secretions into the host, triggering host defense responses. Such responses provide new information for the parasite to make counter-responses. This sequence of responses and counter-responses results in the development of adaptations in behavior, physiology, development, and even morphology for both the parasite and the host (Whitfield, 1979).

Adaptation and Coevolution

Tick Attack Mechanisms

The oral secretions of the tick act as the point of interaction between the tick and its host (Wakelin, 1984). They can be irritating, have hemolytic activity, and cause rapid tissue alterations. They are thought to contain various active reagents responsible for the dilation of skin capillaries, for the development of extensive hemorrhage, for tissue destruction, and for the prevention of blood coagulation in the feeding lesion. The following active agents have been found to exist in tick saliva: anticoagulants, pharmacological agents for vessel dilation (prostaglandins and histamines) and various cytolytic enzymes, such as esterases and phosphatases (Kemp et al., 1982). Histological investigation of tick attachment sites has also shown the presence of lipoprotein-containing

aminopeptidase, associated with the cement, as well as glycoproteins (Walker and Fletcher, 1986). It is important to note that while there is evidence that tick saliva can induce cytolytic reactions, it may also promote autolytic host reactions, causing the host to destroy its own tissues. This may be a natural result of the exposure to the salivary fluids or a strategy utilized by the tick to gain a wider array of food materials (Schleger et al., 1976).

Various paralytic compounds are also sometimes secreted and more than 43 species of tick in 10 genera have been shown to cause paralysis in humans, other mammals, and birds (Gothe et al., 1979). Whether the paralysis is simply a toxic response (Viljoen et al., 1986), an allergenic host response, or has further implications is not well understood at this time.

Host Defense Mechanisms

As reviewed earlier, ticks cause many problems ranging from simple annoyance to the death of their hosts. In theory, those effects may decrease fitness, perhaps even act to regulate host population growth by reducing host reproductive potential (May, 1983). In response, a wide range of host adaptations has evolved enabling the host to minimize the effects of the ticks. These adaptations are known collectively as immunity. Immunity is defined as the sum of all the physiological potential of a host that

protects it from parasite (tick) infestation (Kim, 1985). It may exist in two forms, as an innate or natural resistance, or an acquired response to an initial tick infestation. As will be discussed later, host immunity can be an extremely effective mechanism for the regulation of tick populations.

Tick Counter-Strategies

It is a generally accepted theory that some degree of ecological stability between parasitic arthropods and their natural hosts eventually occurs (Capron et al., 1977). For such stability to occur requires a long period of active evolutionary interactions, and is referred to as adaptation tolerance (Sprent, 1962). The mere presence of modern ticks is evidence that they have somehow developed immune evasion strategies to limit the effects of the immunologic effector mechanisms used by parasitized mammalian hosts (Kim, 1985). In Rhipicephalus sanguineus sanguineus (Latreille), a histamine-blocking agent detected in the salivary gland, regulates the accumulation of histamine that causes the host allergic reaction (Chinery, 1981). Dermacentor andersoni (Stiles) larvae induce a short-lived immune suppression in their host during the attachment period that appears to reduce the host's response to T-cell dependent antigens (Wikel, 1982b, 1985). More recently, anti-hemostatic, anti-inflammatory, and immunosuppressive activities have been demonstrated in the saliva of Ixodes

dammini White (Ribiero, 1987, 1988; Ribiero et al., 1985; Wikel and Whelen, 1986).

Ample evidence exists that ticks and their hosts have undergone a long-term co-adaptation process. Ticks evolved mechanisms to feed on their hosts, as their hosts developed mechanisms to resist those feedings. Finally, ticks appear to have developed counter-strategies to evade the host defense mechanisms. It is a complex series of interactions which, once understood, should enable us to more effectively control ticks and tick-borne diseases.

Tick Control Methods

Present Methods

Due to the close interaction of ixodid ticks with man and man's livestock, almost all tick-control programs have been directed at that group. Two basic strategies have developed: directing control efforts at ticks on the host animal, and attempting to change or treat the environment in which the ticks emerge and search for their host meal.

Control of ticks on the host has been a subject of importance for nearly 100 years. Control efforts in this country directed at the cattle tick, Boophilus spp., the vector of bovine babesiosis, resulted in the discovery of the first tick-borne disease (Smith and Kilbourne, 1893). The acaracide application methods of dips, pour-ons, and spraying, as well as the mechanical methods of picking or brushing ticks off the host, developed at that time are

still utilized today. Many different materials have been used for tick control over the years including sweet or cottonseed oil, carbolic acid, tobacco and or sulphur dips, fish oil, crude petroleum, kerosene, coal tar, arsenical dips, rotenone, and pine tar. More recently, organochlorines, organophosphates, amidines, carbamates, and synthetic pyrethroids have been used both for direct application and systemic action. Sustained release methods such as using a bolus, and the attachment of acaracide impregnated ear tags and wick-like applicators, are the newest application methods being used (Drummond et al., 1988; Miller, 1987). The most recent advance in terms of practical, low-dosage administration of systemics has been obtained with avermictin (Lancaster et al., 1982; Drummond and Miller, 1984), a GABA inhibitor derived from Streptomyces avermitilis (Markell et al., 1986).

Attempts at changing and or treating the off-host environment of the tick also date back a long time. Such methods can be very successful, though expensive, and include treating with acaracides, limited areas of vegetation in which the actively host-seeking ticks are present, rotating animals in and out of pastures in such a way as to eliminate hosts from the ticks during periods of time in which feeding is required, changing the tick's microclimate by burning or otherwise eliminating vegetation that protects the ticks, and by eliminating alternate host animals (Sauer and Hair, 1986). Biological control of

ticks is also of interest, but such agents as anti-tick grasses, parasites, fungi, and other potential biocontrol organisms are presently only in the discovery and evaluation phase (Drummond et al., 1988).

One major problem exists with the past and present usage of acaracides, that of tick resistance to chemical control. As the cost of acaracides continues to rise and the incidence of resistance and cross-resistance increases, alternative control methods will have to be found.

Alternative Control Methods

One alternative is to modify the behavior of the tick to its host (Matthewson, 1984). This can be achieved by the use of activators which prevent the tick from establishing a feeding site or disturbing it once a site has been established. If used in conjunction with an acaracide, an activator might cause the tick to pick up a greater quantity of toxicant and therefore improve the action of the acaracide. Examples would include the use of repellents to make the host unattractive to the tick, and the use of pheromones associated with aggregation and attachment to confuse the ticks.

While the concept of sterile arthropod release for the control of ticks has been proposed (Osburn and Knipling, 1982), it has not yet been tested in the field (Lancaster and Meisch, 1986). Another alternative approach to tick control is the exploitation of host resistance, whether

innate and inheritable (Matthewson, 1984), or acquired as a result of an initial tick infestation or immunization (Wikel and Allen, 1982). Innate host resistance would include the ability of the host to groom itself (i.e., remove ticks), as well as inherited factors such as skin thickness, fur length, and color (George et al., 1985). Such factors can be selected for in a breeding program. Acquired host resistance is defined as the ability of the host to interfere with tick feeding, due to previous tick exposure. It has been shown to occur in laboratory animals and cattle, and is measured in the number of ticks attaching, and a reduction in engorgement weight (Willadsen, 1980). In addition to the decrease in disease transmission caused by reduced numbers of vectors, acquired tick resistance can directly interfere with the transmission of pathogenic organisms (Shapiro et al., 1986).

Host Immune Response Mechanisms to Tick Feeding

Types of Host Immune Responses to Ticks

In general, disease results from the entrance of viral, microbial or parasitic organisms into the body. To defend against this, vertebrates possess a sophisticated screening system, the immune system, which continually screens the blood stream for the presence of foreign or antigenic molecules. Once detected, the invading antigen is identified, and processes for its

elimination are initiated. The immune system is multi-layered and utilizes both immediate and delayed mechanisms for protection (Raven and Johnson, 1987).

The immediate mechanism functions by first isolating the entrance site through the inflammation process (Wakelin, 1984). The immune response then attempts to destroy the invading organism by phagocytosis, utilizing macrophages and granulocytes such as neutrophils, eosinophils, and basophils. The delayed system, consisting of a humoral or B-cell system and a cell-mediated or T-cell system, is turned on by the above phagocytosis, forming antibodies and sensitized lymphocytes; one or both may destroy the invader and subsequent identical invaders (Guyton, 1981). In the case of subsequent exposures this system will also act to accelerate and intensify the immediate response mechanisms mentioned above. So, three interacting response systems exist in mammals: the non-specific cellular response, the B-cell system, and the T-cell system.

In the case of tick infestation, the salivary secretions act as the invading molecules, and it is these molecules to which the host immune response is most likely directed. Several extensive reviews of host immune responses to tick feeding have been completed (Allen, 1987; Kemp et al., 1982; Oberem, 1984; Tatchell, 1987; Wikel, 1982a, 1983, and 1984; Wikel and Allen, 1982; and Willadsen, 1980). The following discussion will cover much

of the same information but from the perspective of the type of mechanism utilized by the host to control tick feeding. Though very little work has been done regarding host immune responses to argasid tick feeding, it will be discussed where information exists.

Immediate Response

When injury occurs, such as that seen during tick feeding, large quantities of histamine, bradykinin, serotonin, and other substances are released by the injured cells into the surrounding tissues. These, especially the histamine, increase the blood flow and the permeability of the venous capillaries and venules, initiating the walling off effect of inflammation. This process stimulates the phenomenon of chemotaxis, through which the three leukocytes primarily involved in the immediate host defense mechanism (neutrophil, eosinophil, and basophil) are attracted to the site and join the macrophages already present.

The neutrophils, which normally make up about 65% of the circulating leukocytes, and macrophages carry out their scavenger functions through the process of phagocytosis, engulfing large amounts of material. Eventually, a cavity may be left filled with pus, a mixture containing dead neutrophils, macrophages, and necrotic tissue. The eosinophils are weak phagocytes, normally making up only 1-3% of the circulating blood leukocytes. They enter the

blood in large numbers after foreign protein injection and have a special ability to phagocytize and digest the combined antigen-antibody complex after the immune system has performed its function. The basophils are normally found in the circulating blood in very low numbers and act in response to injury by releasing heparin, a substance that prevents blood coagulation, and histamine, which causes local vascular and tissue reactions (Guyton, 1981).

Since 1939 (Trager, 1939a), the histology of tick feeding sites on naive and sensitized hosts has been studied in a number of tick-host systems. The results of these studies have revealed that the host reaction to tick feeding is a complex phenomenon and depends greatly on the species of host and tick involved, the time post attachment, and whether the host has been previously exposed. In general, the cellular reactions at tick feeding sites change in character and magnitude with time after attachment, and differ considerably from primary to tertiary infestation (Gill and Walker, 1985).

The primary infestation is marked by infiltration of neutrophils, followed by mononuclear cells and eosinophils. Subsequent infestations differ in that the percentage of basophils infiltrating the bite site significantly increases, causing a complementary decrease in the percentage of neutrophils and eosinophils. Often, an increase in basophil and mast cell (a basophil-like cell) degranulation is also noted. In tick-resistant

guinea pigs, for example, the cellular responses are dominated by basophils and eosinophils (Allen, 1973; Brown and Knapp, 1981; Brown et al., 1983a; Brown and Askenase, 1984). A significant increase in basophils has also been noted in tick-resistant rabbits (Brossard and Fivaz, 1982; Gill and Walker, 1985; Walker and Fletcher, 1986), and cattle (Gill, 1986), though neutrophils still made up the greatest proportion of infiltrating cells.

The importance of basophils in the expression of host resistance to ticks was made evident when anti-basophil serum given to tick-resistant guinea pigs reduced not only the number of basophils and eosinophils, but also ablated the expression of immunity to the ticks (Brown et al., 1982). In another study, genetically mast cell-deficient mice were unable to acquire resistance against larval ixodid ticks (Matsuda et al., 1985). When the same mice were later injected with cultured mast cells, normalization of their anti-tick response occurred (Matsuda et al., 1987). The change in makeup of infiltrating cells as host resistance develops appears to be mediated by several immunological mechanisms, including IgG antibody (Brown and Askenase, 1983), complement (Wikel and Allen, 1977), and T cell mediated responses (Brown and Askenase, 1983).

Work done with argasid ticks has shown that guinea pigs react in much the same way to feeding by Ornithodoros ticks. Subsequent exposures generated increases at the feeding site in both basophils and eosinophils similar to,

if not greater than, those seen in response to ixodid ticks (Brown et al., 1983b; Johnston and Brown, 1985). Despite mounting a strong cutaneous basophil response of the kind that mediates immune rejection of ixodid ticks, no such effects were seen on the argasid ticks; probably a result of their fast feeding nature (McLaren et al., 1983).

The initial, nonspecific response is not designed to eliminate the invading organisms as much as it buys time for the immune response to respond. The key element in this response is a class of T-cells, called helper T-cells, which are stimulated by messages put out by the macrophages active in the initial defense. When T receptors on the surfaces of the activated helper T-cells encounter antigens presented by macrophages or epidermal langerhans cells (Allen et al., 1979: Nithiuthai and Allen, 1985), these cells simultaneously activate two different but parallel systems, the humoral response and the cell-mediated response (Raven and Johnson, 1987).

Hummoral or B-cell Response

The B-cell is the important lymphocyte involved in the humoral response. Upon stimulation by an antigen and a T helper cell, certain lymphokines are released that induce the B-cell to proliferate. After several days of cell division most of the proliferating B-cells stop reproducing and begin to produce many copies of the B receptor protein that initially responded to the antigen. These copies are

secreted as circulating antibodies and may constitute 20% of the total protein in blood plasma by weight (Raven and Johnson, 1987). The major properties of these secreted antibodies are: complement fixation, by either the classical or alternate pathway, opsonic activity (attraction of neutrophils, eosinophils, and basophils), attachment to mast cells and basophils, macrophage binding, and placental passage regulation (Kehoe, 1978). Antibodies may be involved in both immediate and delayed hypersensitivity reactions.

A great deal of evidence exists that tick feeding induces a measureable host antibody response. That response at the very least indicates that tick feeding has occurred, an important factor in determining disease transmission parameters (J.F. Butler, pers. com.). More importantly, it may be a key factor in the acquisition of tick resistance in host animals.

Passive transfer experiments with whole plasma and immune serum from previously infested and tick-resistant hosts induced tick resistance in naive host animals (Roberts and Kerr, 1976; Askenase et al., 1982). Further indirect evidence that tick resistance may be antibody mediated was obtained from experiments in which guinea pigs treated with immunosuppressants did not express resistance upon sequential exposure, while control animals did (Wikel and Allen, 1976).

One mechanism for antibody-induced resistance appears to be antibody induced effector cell (basophils and eosinophils) recruitment (Askenase et al., 1976; Brown and Askenase, 1983, 1985b). Fractionation of immune serum by gel filtration and ion exchange chromatography, and testing in vivo, has shown that antibodies mediating resistance are in the IgG and IgG1-containing peaks (Brown and Askenase, 1984). Furthermore, antibody Fc receptors on host cells such as mast cells and basophils have been shown to be required for antibody-mediated immune rejection of ticks (Brown and Askenase, 1985a). Finally, some evidence for both complement and IgE-like anaphylactic activity has been reported, though it is only circumstantial at this time (Willadsen, 1980; Willadsen et al., 1979).

As stated earlier, tick salivary secretions injected during feeding act as the inducers of the host antibody response. Several studies with host antibody and Western blotting techniques have shown this to be true and isolation of the specific proteins responsible for the host resistance has been conducted (Brown, 1986; Brown and Askenase, 1986a, 1986b; Brown et al., 1984; Gill et al., 1986; Gordon and Allen, 1987; Shapiro et al., 1986; Whelen et al., 1986; Wikel and Whelen, 1986). The isolation of proteins from tick salivary glands that induce host resistance is presently a very active area of research and will be discussed in more detail later.

Several studies indicate that not only do host animals express a strong acquired resistance to one species of tick, but that resistance is cross-reactive to other species and genera of ixodid ticks to which the host has not been exposed. In addition, one study has shown that certain proteins in mites may be cross-reactive with ticks (denHollander and Allen, 1986). This may indicate shared antigens among these hematophagous arthropods that are important in the induction of host resistance (Brown and Askenase, 1984).

Cell-Mediated or T-cell Response

The T helper cells that initiate the humoral response also initiate a series of events that are known as the cell-mediated immune response. Once stimulated by soluble factors released by phagocytosis, the T helper cells begin secretion on their own. Most important of these secretions is interlukin-2, or T-cell growth factor. Under its influence T-cells that are in contact with antigen begin proliferation, forming large clones of T cells capable of recognizing that antigen. Three T cell types proliferate: helper T cells, cytotoxic killer cells, and suppressor T cells, each with its own specific function. These T cells interact to assist the antibody response, traffic throughout the body to recognize and attack foreign antigen through cell membrane disruption, and then slow down and control the host immune response after the infection has

been checked (Raven and Johnson, 1987). These responses are also referred to as delayed hypersensitivity reactions.

Evidence showing the involvement of T cells in the expression of host resistance is not as clear because this area has not been as thoroughly investigated. Acquired resistance has been transferred from resistant donors to naive recipients with viable lymph node cells or peritoneal exudate cells (Askenase et al., 1982; Brown and Askenase, 1981), indicating T-cell-mediated involvement. In vitro work with lymph node proliferation responses to tick salivary antigens from guinea pigs undergoing first and second infestations also supports the idea that T cells are involved in the development of acquired host resistance (Wikel et al., 1978). One mechanism responsible for T cell induced resistance (delayed hypersensitivity) appears to be activation of mast cells by antigen-specific T cells that in turn, initiate T cell recruitment of effector cells (Askenase and Van Loveren, 1983).

Effects of the Host Immune Response On Ticks

The development of acquired host resistance to ixodid tick feeding in response to an initial tick infestation was first noted in cattle many years ago (Johnston and Bancroft, 1918). Since that time it has been shown to develop in guinea pigs, rabbits, rats, mice and other wild rodents, sheep, horses, dogs, and possibly some amphibians.

In general, the manifestation of acquired resistance results in some or all of the following effects depending on the species of host and tick involved: reduced numbers of ticks feeding to repletion in a challenge infestation, reduced weights of fed ticks, reduced feeding rates, reduced ability of fed larvae or nymphs to molt to the next life stage, and reduced progeny of fed female ticks (Allen, 1987).

In laboratory tests, argasid tick feeding does stimulate host immune responses, but those responses occur after the feeding has been completed, and have no effect on the ticks (Brossard et al., 1981; Brown et al., 1983b; McLaren et al., 1983; Trager, 1940). One exception occurred in measurements of the effects of sequential feedings of Argas persicus larvae, which take at least four days for engorgement, on chickens. Partial immunity was noted and was apparently due to the longer attachment and feeding period (Trager, 1940). Another exception should be considered. If the host is a natural inhabitant of the burrow in which the argasid ticks live, that host would be under constant exposure to argasid tick feeding and would therefore always be presenting a defensive immunological response (J.F. Butler, pers. com.). Under such conditions, those ticks would be feeding on hosts that could have a significant negative effect on tick survival.

When acquired resistance does occur, various mechanisms may be at work. The recruitment of effector

cells such as eosinophils and basophils to a feeding site in resistant animals appears to have serious consequences for the feeding tick. The components of the host response causing these effects are not known, but possibly include degranulation products from the effector cells. Investigation of the effects of histamine on the attachment of Boophilus microplus larvae has shown that the concentration of histamine is much higher in tick-resistant animals (Allen and Kemp, 1982). Whether the histamine is involved in host discomfort that causes itching and tick removal, or local anaphylaxis reactions, is not known (Kemp and Bourne, 1980). The most severe effect appears to be on the gut stem cells of the tick which are, for some reason, unable to proliferate or differentiate adequately (Walker and Fletcher, 1987). The resulting hypoplasia prevents the gut from accomodating a blood meal at final engorgement of equal size as that taken by ticks feeding on naive hosts.

The uptake of host antibody during feeding and its passage into the tick hemolymph is another possible mechanism and has been noted in several ixodid (Ackerman et al., 1981; Ben-Yakir et al., 1986) and argasid ticks (Chinzei and Minoura, 1988; Minoura et al., 1985). The role of these antibodies in the development of acquired resistance is not known but has been suggested to include inhibition and neutralization of tick enzymes and hormones (Ben-Yakir et al., 1986). The use of antitick-antibodies obtained through host immunization using specific tick

antigens is a very interesting and exciting field of interest at this time.

Applications of the Host Immune Response for Tick Control

Two basic approaches have developed utilizing the host's immune response as a means for controlling ticks (Willadsen, 1987). The first approach involves exploitation of the fact that hosts acquire an immunologically mediated resistance after repeated tick infestations that limits further feeding. In this case the host responds to proteins injected during feeding or to vaccinations of tick salivary gland that simulate that feeding. The host-tick interface is the site of immunomodulation and the host animal basically rejects the tick. A minimum of 24-48 hours is generally required before the host response occurs, so this method of control is only effective against those ticks which remain attached for several days. This has been attempted with several species of host and tick and has shown promising results (Trager, 1939b; Wikel, 1981; Brown et al., 1984).

The second approach involves the use of isolated tick antigens, those with which the host would not normally come in contact such as tick gut, to induce host resistance (activate host antibody) by direct immunization. The site of antibody action is internal to the tick. Activated host antibody taken up in the bloodmeal binds to its's antigenic counterpart, hopefully disrupting cellular function and

increasing tick mortality. Theoretically, this could be used against both ixodid ticks and the fast feeding argasid ticks which do not remain attached long enough to suffer the consequences of the host's cellular response.

This approach was first shown to be of great potential as a means of mosquito control in 1972, (Algar and Cabrera, 1972). Anopheles stephensi mosquitoes that were fed on hosts that had been immunized with mosquito midgut preparations sufferred a significantly higher mortality rate as compared to mosquitoes feeding on control animals. Similar results were found with Stomyxs calcitrans (Schlein and Lewis, 1976). It was first proposed as a concept for tick control in 1975 (Galun, 1975), and successfully carried out in 1979 (Allen and Humphreys, 1979). Ticks fed on guinea pigs that had been immunized with tick internal organs suffered drastic reduction in engorgement weight and a significant reduction in egg laying. Since that time artificially induced, partial immunity has been shown in three host species against five ixodid (Willadsen, 1987), and one argasid tick species (Chinzei and Minoura, 1988).

Overview and Statement of Objectives

In the late 1970s, the introduction of African swine fever to the Caribbean and the possibility it could be introduced into the United States awakened a renewed interest in the study of the potential vector argasid ticks in this country (Milstrey, 1987). Since that time numerous

studies regarding Florida argasid ticks have been completed (Adeyeye, 1982; Beck et al., 1986; Butler et al., 1984a, 1984b, 1985; Endris et al., 1986; Milstrey, 1987; Telford, unpublished data; Wozniak, in manuscript). As work progressed a lack of knowledge involving the host immune response to argasid tick feeding became quite obvious. While a great deal of research has been completed regarding ixodid ticks and their host-immune response interactions, very little has been studied regarding argasid ticks and their host-immune response interactions.

In this chapter, an attempt has been made to review the superfamily Ixodoidea, introducing the morphological, biological, developmental, and behavioral similarities and differences between ixodid and argasid ticks. The medical and veterinary importance of ticks as well as the evolutionary history of ticks and their hosts has also been discussed. Finally, the host immune response to ixodid tick feeding, its effect on ticks, and its possible application as a control strategy has been presented.

The purpose of this research was to fill in some of the gaps regarding argasid ticks and their host-immune response interactions. The primary objectives were as follows:

1. to determine the effects of sequential host feedings on argasid tick survival, weight gain, and attachment time;

2. to determine host antibody responses to sequential feedings;

3. to determine protein characterization of argasid tick salivary glands with emphasis on identifying the specific proteins responsible for eliciting the host antibody response;

4. to conduct histological and *in vitro* studies of the host cellular responses to sequential argasid tick feedings;

5. to propose and test possible applications of the host immune response as a control mechanism for argasid ticks.

CHAPTER 2
SEQUENTIAL FEEDINGS BY TWO SPECIES OF ARGASID TICK ON LABORATORY MICE; EFFECTS ON TICK SURVIVAL, WEIGHT GAIN, AND ATTACHMENT TIME

Introduction

Ticks cause many problems for their host ranging from simple annoyance to death. In theory, those effects may decrease fitness, perhaps even act to regulate host population growth by reducing host reproductive potential (May, 1983). In response, a wide range of host adaptations have evolved enabling the host to minimize the effects of the ticks. These adaptations are known collectively as immunity, which is defined as the sum of all the physiological potential of a host that prevents it from parasite (tick) infestation (Kim, 1985). It may exist in two forms, as an innate or natural resistance, or an immunologically acquired response to an initial tick infestation.

The development of acquired host resistance to ixodid tick feeding in response to an initial tick infestation was first noted in cattle many years ago (Johnston and Bancroft, 1918). Since that time it has been shown to develop in guinea pigs, rabbits, rats, mice and other wild rodents, sheep, horses, dogs, and perhaps some amphibians. In

general, the manifestation of acquired resistance results in some or all of the following effects depending on the species of host and tick involved: reduced numbers of ticks feeding to repletion in a challenge infestation, reduced weights of fed ticks, reduced feeding rates, reduced ability of fed larvae or nymphs to molt to the next lifestage, and reduced progeny of fed female ticks (Allen, 1987).

The mechanisms involved in host acquired resistance causing the above manifestations are numerous and may include both antigen dependent T-cell and B-cell effector cell recruitment and degranulation (Askenase et al., 1976; Brown and Askenase, 1983, 1985), uptake of host antibody during feeding (Ackerman et al., 1981; Ben-Yakir et al., 1986), complement activity (Willadsen, 1980) and IgE-like anaphylactic activity (Willadsen et al., 1979). While argasid tick feeding does stimulate host immune responses, those responses are believed to occur after the feeding has been completed, having no effect on the ticks (Brossard et al., 1981; Brown et al., 1983b; Mclaren et al., 1983; Seubert, 1978; Trager, 1940). One exception was noted when measuring the effects of sequential feedings of Argas persicus larvae, which take at least four days for engorgement, on chickens. Partial immunity was noted, apparently due to the longer attachment and feeding period (Trager, 1940).

Preliminary observations made during colony maintenance of argasid ticks led us to suspect that the host immune

response of the laboratory mice used for feeding tick colonies might be causing decreased tick survival. The objective of this study was to determine if the immune responses of laboratory mice to sequential feedings of two species of argasid tick with different feeding strategies, would affect tick survival, weight gain at repletion, and the time the ticks remained attached to their host.

Materials and Methods

Two species of Ornithodoros ticks were maintained in colony for the experimental feeding trials. One species, O. turicata (Duges) (originally collected from Texas), feeds in less than one hour in all instars (Davis, 1941), typical of many argasid ticks. The other species, O. talaje (Guerin-Meneville) (from nests of the Key Largo Woodrat, Neotoma floridana smalli Sherman), attaches for six to eight days during its larval feeding stage but feeds similarly to O. turicata at the other life stages (Cooley and Kohls, 1944).

The colony ticks were maintained at 26° C. and ca. 85% RH in a Hotpack temperature-humidity chamber (Model 435310, Hotpack Corp., Philadelphia, PA). Except for feeding and observation, the colony ticks were maintained in total darkness. Hosts for normal colony feeding were CD1 mice. While laboratory mice are obviously not one of the natural hosts, both tick species naturally feed on wood rats and other rodents which live in the burrows in which the ticks were originally found (Milstrey, 1987). Furthermore, both

tick species have been shown to survive and reproduce quite effectively using laboratory mice for host feeding (Beck et al., 1986). Additional details for normal tick rearing procedures can be found in the above citation. Host mice were reared in a separate room under a 12:12 day-night regime, at 28° C. and ca. 70% RH. Day to day care was provided under contract by Animal Resources, IFAS, Univ of Florida.

For this study, host exposure to O. turicata larvae was set up in the following manner. On three separate occasions, once every thirty days, twenty O. turicata larvae were permitted to feed on individual (10) 2 month old, female CD1 mice. The first exposure was on naive mice; the second and third exposures were to the same, now previously exposed mice. The larvae were exposed to the mouse for 1.5 to 2 hours through the mesh screen of the lid of a plaster-bottomed 125 ml plastic container that had been temporarily taped to the belly of the mouse.

Host exposure to O. talaje larvae was set up as follows. On two separate occasions, once every thirty days, O. talaje larvae were permitted to feed on individual (10) 2 month old, female CD1 mice. Due to mortality in one case, and recurring eye infections in three other cases, only six of the ten mice were exposed to a third feeding. Again, the first exposure was to naive mice while the second and third exposures were to the same, now previously exposed mice. The slow feeding characteristics of this species required

the mice to be anesthetized and collared with a plastic device to prevent normal grooming of ticks prior to repletion. Twenty O. talaje larvae were individually placed on each mouse. The mice were then put in separate screened plastic boxes with adequate food and water and held under normal room conditions for six to ten days until ticks were replete and had detached.

At completion of feeding by both species of tick, the ticks were immediately weighed using a Mettler balance (Model E-200). Due to the small size of the fed O. turicata it was necessary to weigh the entire group of larvae from one mouse and then divide the weight by the number of ticks feeding to obtain an estimate of individual tick weights. Individual weights as well as the number of days to repletion of the O. talaje larvae were noted. Finally, survival of the individual larval ticks of both species was measured. Survival was defined as those individuals feeding and molting to the nymphal stage. Statistical analysis of all results was conducted using a data analysis package (RS1 Integrated Data Analysis) designed for a Digital Professional 350 computer.

Results

Survival of O. turicata larvae, which feed quickly, did not significantly ($p>.05$) change upon sequential host exposure, remaining at more than 98% after all three feedings. Survival of the O. talaje larvae, which remain

attached for 6-8 days was significantly altered (@ $p<.05$). Survival decreased from 78% on naive mice to 71% on second exposure to 58% upon third exposure. Table 2.1 lists the numbers and percent survival of both species of tick recovered from the three feedings on mice. While the decrease in percent survival between the first and second exposure of O. talaje larvae was not significant at the .05 level (Chi square, 1 DF, =2.86), it was significant at the 0.1 level. The decrease in survival between the first and third exposures was, however, highly significant (Chi square, 1DF, .001 level= 23.3). If the decrease in survival is considered from the perspective of increased mortality, Figure 2.1 clearly shows the effect of sequential host exposures and the development of acquired host resistance on O. talaje larvae.

While survival of O. turicata larvae to the nymphal stage was not significantly affected by sequential host exposures, their detachment weight was affected. A highly significant ($p=.001$) decrease in detachment weight from a mean of 0.488 to 0.409 mg occurred between both the first and second host exposure (t value, 18 DF, =4.23). The decrease in mean detachment weight from 0.488 to 0.386 mgs in the first and third exposures was also highly significant (t value, 18 DF, =4.08). Detachment weight of individual O. talaje larvae, on the other hand, did not change significantly between first, second and third exposures. Table 2.2 lists the weight in milligrams of detaching larvae

of both species of tick recovered from the three feedings on mice. Figure 2.2 illustrates the average detachment weight of O. turicata larvae resulting from three sequential feedings.

As can be seen in Table 2.3, an increase from 7.2 to 7.6 days occurred in the amount of time O. talaje larvae remained attached to their host upon sequential exposure. The difference in time attached between the first and second exposures was significant at the .05 level (z value, 275 DF, = 2.17), though the difference between the first and third exposures was significant only at the .1 level (z value, 243 DF, = 1.67).

Discussion

Very little research has been conducted regarding host immune effects on argasid ticks. This is in part due to the generally accepted hypothesis that because argasid ticks feed so rapidly, the host can not respond quickly enough immunologically to interfere with feeding. Certain facts learned in this study may lead to a modification of the above hypothesis.

The finding that the long feeding O. talaje larvae suffered increased mortality upon second and third host exposures, while rarely noted for argasid ticks, is not surprising. As cited earlier (Trager,1940), acquired host resistance to long feeding argasid ticks has been reported. It is interesting to speculate about the host resistance

Table 2.1. Numbers (percent) of surviving larvae of two species of Ornithodoros tick recovered from three sequential feedings on laboratory mice.

Tick Species	First Exposure	Second Exposure	Third Exposure
O. turicata	189/193 (98%)	163/165 (98%)	161/162 (99%)
O. talaje	125/160 (78%)	128/180 (71%)	70/120 (58%)

Table 2.2. Average weight in milligrams of detaching larvae of two species of Ornithodoros tick recovered from three sequential feedings on laboratory mice.

Tick Species	First Exposure	Second Exposure	Third Exposure
O. turicata	.488	.409	.386
O. talaje	1.997	2.160	2.100

Table 2.3. Average number of days Ornithodoros talaje larvae remained attached to their host during three sequential feedings on laboratory mice.

First Exposure	Second Exposure	Third Exposure
7.3	7.6	7.6

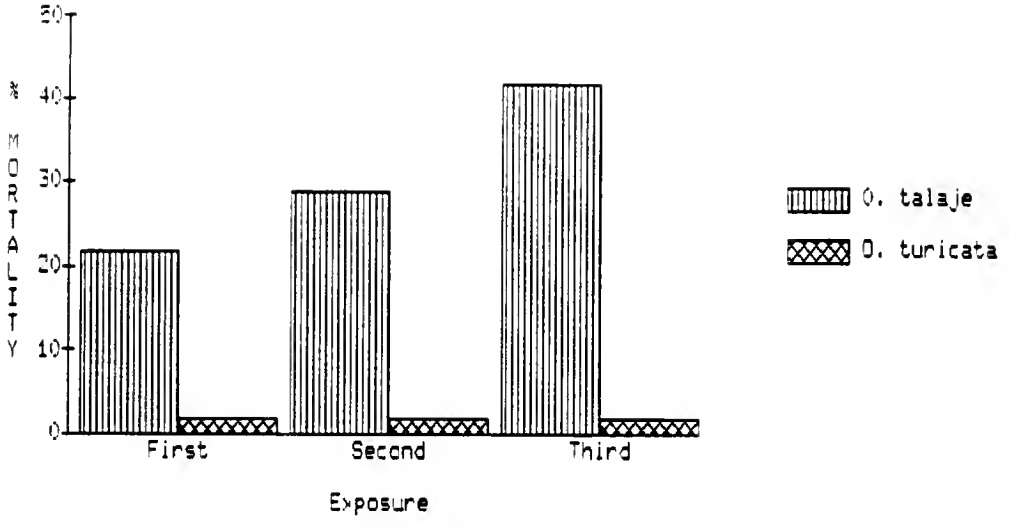

Figure 2.1. Average tick mortality resulting from three sequential feedings on laboratory mice.

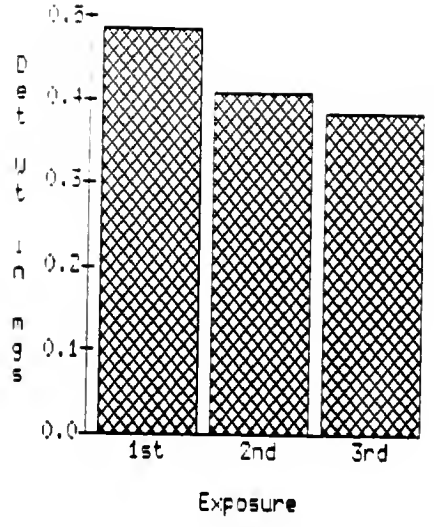

Figure 2.2. Average detachment weight of O. turicata larvae resulting from three sequential feedings on laboratory mice.

mechanism causing the increased mortality because as the amount of time the larvae remained attached to the host increased significantly upon sequential feedings, the detachment weight did not. Perhaps interference with the feeding process at the feeding site is occurring. An alternative is that something taken up in the blood meal, either host antibody or degranulation products, is slowing the feeding or digestive process.

The most surprising finding is that though sequential host exposures did not effect the immediate survival of the fast feeding O. turicata larvae, it had a very significant effect on the amount of weight individual ticks gained during each feeding. The long range effect of a series of smaller blood meals from sensitized hosts on later argasid tick development and production of offspring may be considerable. It is interesting to consider that the only host resistance mechanism that could respond quickly enough to affect the amount of weight gained during a 60 minute feeding period is that of an allergic nature. Allergic responses in mice may be mediated by either IgE or IgG1 antibody (Spiegelberg, 1974; Voisin, 1976), and work reported in chapter 3 shows that these same mice had elevated anti-tick-salivary-gland IgG1 antibody levels after only 2 exposures.

One should also consider that only three sequential host exposures were used in this study. If the host were a natural inhabitant of the burrow in which the argasid ticks

live, it would be under constant exposure to argasid tick feeding. In response, it would constantly maintain a defensive immunological posture which could have a very significant negative effect on tick survival, perhaps to the point of actually limiting field populations of these parasites (J.F. Butler, pers. com.). This is a concept that should certainly receive further investigation.

In summary, acquired host resistance does develop in CD1 mice as a result of sequential exposures to 20 larvae of two species of Ornithodoros tick. In the case of the fast feeding O. turicata, the average amount of weight gained during feeding decreased significantly the second and third times larvae were exposed to the same mice. In the case of the O. talaje larvae, which take from 6 to 8 days to feed, mortality upon second and third exposure significantly increased when compared to that seen upon naive mice. In addition, while attachment time increased, detachment weight was not effected. This study provides evidence then, that acquired host resistance in mice does develop and it appears to affect argasid ticks, even those that feed very rapidly.

CHAPTER 3
ANTIBODY RESPONSES OF LABORATORY MICE TO SEQUENTIAL FEEDINGS BY TWO SPECIES OF ARGASID TICK

Introduction

A great deal of evidence exists that ixodid tick feeding induces a measureable host antibody response. That response at the very least marks the fact that tick feeding has occurred, an important factor in determining disease transmission parameters (J.F. Butler, pers. com.). More importantly, it may be a key factor in the acquisition of host resistance to tick feeding.

Passive transfer experiments with whole plasma and immune serum from previously infested and tick-resistant hosts have shown induced tick resistance in naive host animals (Roberts and Kerr, 1976; Askenase et al., 1982). Further indirect evidence that tick resistance may be antibody mediated was obtained from experiments in which guinea pigs treated with immunosuppressants did not express resistance upon sequential tick exposure, while control animals did (Wikel and Allen, 1976).

Tick salivary secretions during feeding act as the inducers of the host antibody response. Several studies using host antibody and Western blotting techniques have shown this to be true and isolation of the specific proteins

responsible for inducing the host antibody response and possibly responsible for the acquisition of host resistance to tick feeding have been conducted (Brown, 1986; Brown and Askenase, 1986a, 1986b; Brown et al., 1984; Gill et al., 1986; Gordon and Allen, 1987; Shapiro et al., 1986; Whelen et al., 1986; Wikel and Whelen, 1986).

One mechanism for antibody-motivated resistance appears to be antibody induced effector cell (neutrophil, basophil and eosinophil) recruitment (Askenase et al., 1976; Brown and Askenase, 1983,1985a). Fractionation of immune serum and testing in vivo have shown that the antibodies mediating resistance in guinea pigs are in the IgG and IgG1 containing peaks (Brown and Askenase, 1984). Furthermore, antibody Fc receptors on host cells such as mast cells and basophils are required for antibody-mediated immune rejection of ticks (Brown and Askenase, 1985a). Finally, some evidence for both complement and IgE- like anaphylactic activity has been reported (Willadsen, 1980; Willadsen et al., 1979).

Several studies indicate that not only do host animals express a strong acquired resistance to one species of tick, but that resistance is cross-reactive to other species and genera of ixodid ticks to which the host has not been exposed (Labarthe et al., 1985). Additional work has shown that certain proteins in mites may be cross-reactive with ticks (denHollander and Allen, 1986). This may indicate shared antigens among these hematophagous arthropods that

are important in the induction of the host antibody response and the acquisition of host resistance to tick feeding (Brown and Askenase, 1984).

To date, very little work has been conducted regarding the development of host resistance to argasid tick feeding. Chapter 2 presents evidence that acquired host resistance to argasid tick feeding can develop in mice and speculates that it may be antibody mediated. The purpose of this study was to measure and isotype the host antibody response of mice to sequential exposures of two species of Ornithodoros tick in an effort to determine the mechanism for the development of acquired resistance as well as determine if the host antibody response to fast and slow feeding argasid ticks differed. In addition, an effort was made to determine if the salivary proteins inducing an antibody response to one species of Ornithodoros tick were cross-reactive with those of other tick species.

Materials and Methods

Antibody Isotyping

Two species of Ornithodoros ticks were maintained in colony for use in this study. One species, O. turicata feeds in less than one hour in all instars (Davis, 1941), typical of many argasid ticks. The other species, O. talaje, attaches for six to eight days during its larval feeding stage but feeds similarly to O. turicata at the other life stages (Cooley and Kohls, 1944). The colony

ticks and laboratory mice were maintained as discussed in Chapter 2.

For this study host exposure to the two species of tick was set up in the following manner. On three separate occasions, once every thirty days, twenty larvae of each tick species were permitted to feed on individual, 2 month old, female CD1 mice (3 mice, each tick species). The first exposure was on naive mice; the second and third exposures were to the same, now previously exposed mice. Exposure procedures are discussed in Chapter 2.

As the sequential host exposures were carried out the host antibody response to tick feeding was measured and isotyped. Approximately 100 ul of blood were taken weekly from the six individual mice via standard tail-veining procedures. The blood samples were centrifuged for several minutes (@3000 g) using Microtainer brand serum separators, and the serum samples were diluted 1:50 in 0.15 M potassium phosphate buffered saline, pH 7.2 (PBS)(made as described in Voller et al., 1980). An enzyme linked immunosorbent assay (ELISA) using antibody isotype specific alkaline-phosphatase linked antimouse (Fisher) was conducted following standard procedures (Voller et al., 1980). The antibody isotyping procedural steps are outlined in Figure 3.1. This method is used to determine the class and subclass of mouse immunoglobulin response and the following were measured; total immunoglobulin (IgG, IgM, light and heavy chains) as well as the individual IgM, IgG1, IgG2A, IgG2B, and IgG3

(1) Antigen (tick salivary gland, 3ug) bound overnight to plate.

Block, 1hr. (FCS and BSA)
Wash, 3xs. (PBS/Tween 20)

(2) Mouse anti-tick serum (1:50) added. Specific antibody binds antigen.

Wash, 3xs

(3) Goat anti-mouse isotype (Fc) specific (i.e. IgG1, IgG2..) alkaline phosphatase-linked antibody, attaches to mouse antibody.

Wash, 3xs

(4) 50 ul of substrate added; if enzyme bound, color appears. Amount hydrolyzed = amount of that isotype of tick specific mouse antibody present.

Figure 3.1. Diagram of the procedural steps involved in alkaline phosphatase linked ELISA isotyping of mouse antibody. (Modified from Voller et al., 1980)

isotypes. Serum from exposed mice as well as serum from naive mice were used as positive and negative controls, respectively.

Attempts at isolating sufficient tick salivary fluid for use as an ELISA antigen source were not successful. Therefore, ticks of both species were glued (Superglue brand) ventral side down on disposable petri dishes, immersed in PBS, and the whole salivary glands were removed, rinsed and placed in PBS. The dissected salivary glands were then sonicated, diluted in carbonate-bicarbonate coating buffer (pH 9.6), and used as an antigen source. Fifty uls of antigen solution per well (2.5 ugs antigen/ well) were bound overnight in a refrigerator using Immunulon 2 brand microtiter plates. Protein determination was made via standard procedures (Bradford, 1976) using known amounts of bovine serum albumin for comparison. The next morning blocking was conducted for one hour using 3% fetal calf serum/1% bovine serum albumin in PBS, and the primary and secondary antibodies were each incubated at room temperature for at least 60 minutes. Three rinses using PBS/.5% Tween 20 were made between each step. After 50 uls (1mg/ml) per well of color substrate (p-nitrophenyl phosphate, disodium salt) were applied, the microtiter plates were permitted to develop for at least 60 minutes at 39^{o}C, stopped using 4 M NaOH, and read immediately at 405 nm on a Cambridge micro-plate reader (Model 700). Absorption readings for exposed serum were divided by those for naive mouse serum to control

for background binding. Values above two are considered significant (Voller et al., 1980).

Cross-Reactivity Trial

Preliminary attempts (not reported) to determine if argasid tick feeding would induce a measureable host antibody response were conducted using standard radioimmunoassay (RIA) techniques (Segal and Klinman, 1976). Tick salivary gland antigen was bound in flat bottomed plastic microtiter plates (Falcon Diagnostics, Pittsburg PA), incubated with primary and secondary antibodies, and rinsed as described above, except that a radio-isotope labeled anti-mouse antibody (Sigma) was utilized as the secondary antibody. Detection of anti-tick salivary gland antibody was accomplished by the addition of 25 ul of affinity purified goat anti-mouse IgG, IgM, light and heavy chain immunoglobulin diluted in PBS. That immunoglobulin was iodinated with 1 mCi of carrier free ^{125}I (New England Nuclear, N. Billerica, NY), by a modification of the Iodogen method of Markwell and Fox (1978). Specific activity of 0.7-0.9 uCi/ugm immunoglobulin were obtained. The plates were read immediately on a LKB Wallac gamma counter (Model 1275).

A cross-reactivity test using these RIA methods was conducted with serum from mice that had been exposed three times to either O. turicata or O. talaje larvae. Each serum (diluted 1:50 in PBS) was incubated with 2.5ug/well of tick

antigen (sonicated salivary glands) from three tick species, O. talaje, O. turicata, and an ixodid tick, Dermacentor variablis (Say). Radioactivity counts for the exposed serum were divided by counts for naive mouse serum. Again, values above two are considered significant and indicate an antibody response to that antigen.

Results

Antibody Isotyping

Tables 3.1 and 3.2 present the average corrected ELISA absorbance values for the total immunoglobulin (IgG, IgM, light and heavy chain) response as well as for each of the antibody isotypes of mice exposed to O. turicata and O. talaje larvae, respectively. Figure 3.2 displays the total immunoglobulin (IgG, IgM, light and heavy chain) response to the three sequential exposures of both species of tick without regard to antibody class and subclass. Figures 3.3-3.7 display and compare the individual mouse antibody class and subclass responses to the three sequential exposures of both species of tick.

No significant antibody responses were noted in mice exposed for the first time to either tick species. Upon second exposure significant (values >2x above control serum) responses were noted to feeding by both tick species in total immunoglobulin, as well as the IgM and IgG1 isotypes. After the third exposure significant responses were noted in only the IgG1 class and could be detected for at least 90

days after the last exposure. In general, the degree of response did not appear to differ between the fast and slow feeding species.

Cross-Reactivity Trial

Table 3.3 presents the radioimmunoassay results of the cross-reactivity trial. Serum from O. turicata exposed mice showed the highest affinity for salivary glands (SG) from the same species. Serum from O. talaje exposed mice showed greater affinity for O. turicata SG than for the species to which they had been exposed. In fact, that serum showed greater affinity for SG proteins of an entirely different family of ticks than it did for the species for which it had been exposed. It appears that there is at least one cross-reactive protein in the salivary glands of the three tick species examined in this experiment. Furthermore, there is more of the cross-reactive antigen present in salivary glands of O. turicata and D. variablis than in those of O. talaje.

Discussion

It is not surprising that mice exposed to ticks for the first time showed no antibody response. The amount of antigen or saliva injected by even 20 larvae is low and it was the first or primary exposure. The total immunoglobulin (Ig) response was significant upon second exposure, but it was not significant after the third exposure. This can be

Table 3.1. ELISA antibody isotype results of three mice exposed on three occasions to O. turicata larvae. (Absorbance values for exposed serum/control serum, values shown are average values for 3 mice).

Number of days after exposure	ABS Values					
	Ig	IgM	IgG1	IgG2A	IgG2B	IgG3
First						
+7	1.17	.76	.25	.25	.99	1.9
+14	1.00	.78	1.25	1.06	1.14	1.08
+21	1.31	.79	1.52	1.12	1.45	1.06
+30	1.24	.92	1.93	1.04	1.27	1.09
Second						
+10	6.90	3.50	12.50	1.00	1.00	1.00
+14	2.30	3.60	3.18	.99	.73	.62
+21	2.80	1.70	6.30	1.00	3.20	2.80
+28	1.67	1.76	4.31	1.13	1.08	1.74
Third						
+14	1.24	.98	4.68	1.20	3.02	2.07
+21	1.02	.65	7.11	.25	1.77	.92
+28	.65	.46	7.70	.25	.50	1.00

Table 3.2. ELISA antibody isotype results of three mice exposed on three occasions to O. talaje larvae. (Absorbance values for exposed serum/control serum, values shown are average values for 3 mice).

Number of days after exposure	ABS Values					
	Ig	IgM	IgG1	IgG2A	IgG2B	IgG3
First						
+7	.82	.71	1.39	.61	1.91	.33
+14	1.12	1.00	1.36	1.13	.90	.93
+21	1.03	1.19	1.21	1.21	1.11	1.06
+28	1.11	.95	1.53	1.00	1.11	.97
Second						
+10	3.00	3.40	4.70	1.00	1.00	1.00
+14	1.86	3.25	1.6	1.23	2.32	1.50
+21	6.19	3.99	9.47	1.00	1.00	1.00
+28	1.89	2.85	.96	1.32	1.04	1.10
Third						
+14	.82	1.01	.98	1.07	1.07	1.12
+21	.64	.92	2.56	.62	1.49	.76
+28	.69	1.44	2.37	.50	.30	.90

Table 3.3. RIA results showing degree of cross-reactivity between salivary gland proteins of three species of tick.

Type of serum - type of salivary gland	RIA Result
O. turicata exposed mouse serum with *O. turicata* salivary gland	8.6
O. talaje exposed mouse serum with *O. turicata* salivary gland	5.4
O. talaje exposed mouse serum with *D. variablis* salivary gland	4.2
O. talaje exposed mouse serum with *O. talaje* salivary gland	3.5
O. turicata exposed mouse serum with *D. variablis* salivary gland	3.1
O. turicata exposed mouse serum with *O. talaje* salivary gland	1.7

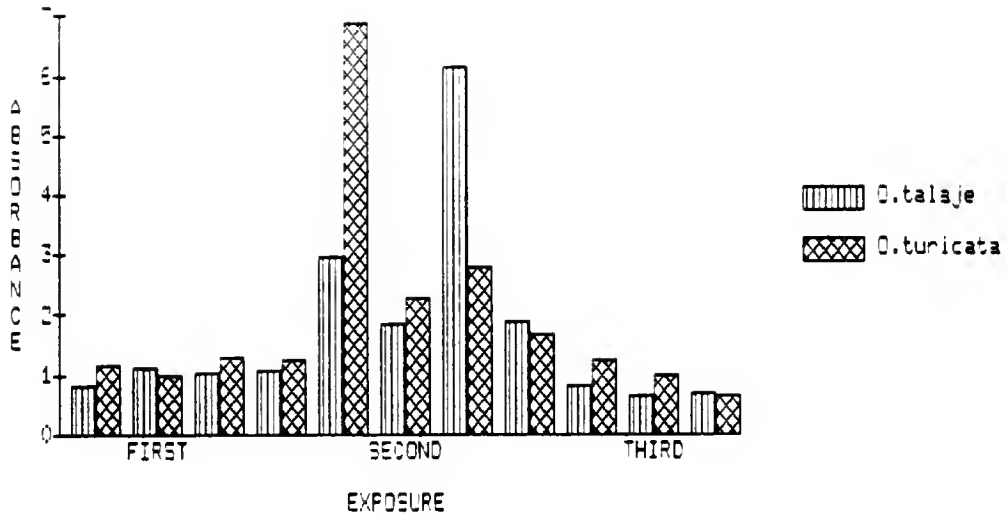

Figure 3.2. Mouse Ig response to three exposures of two species of Ornithodoros tick.

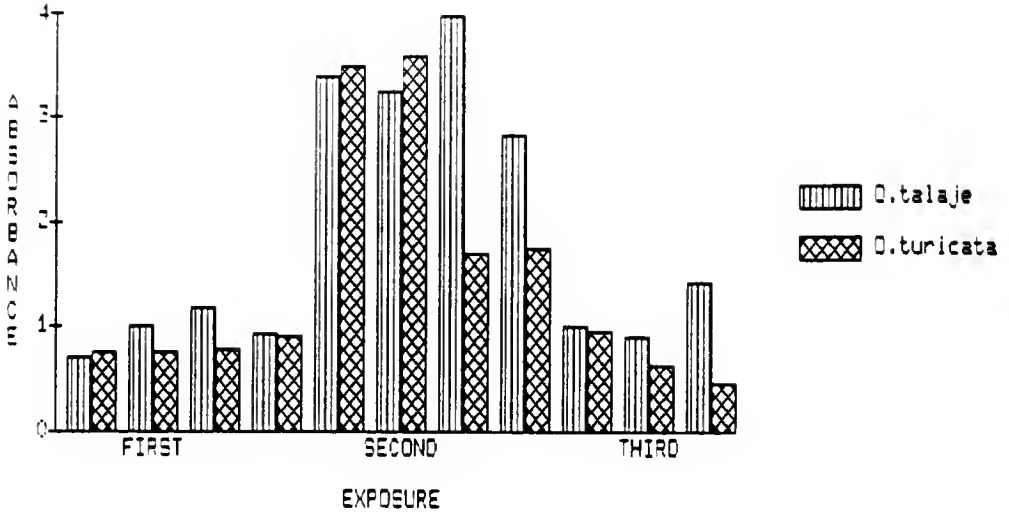

Figure 3.3. Mouse IgM response to three exposures of two species of Ornithodoros tick.

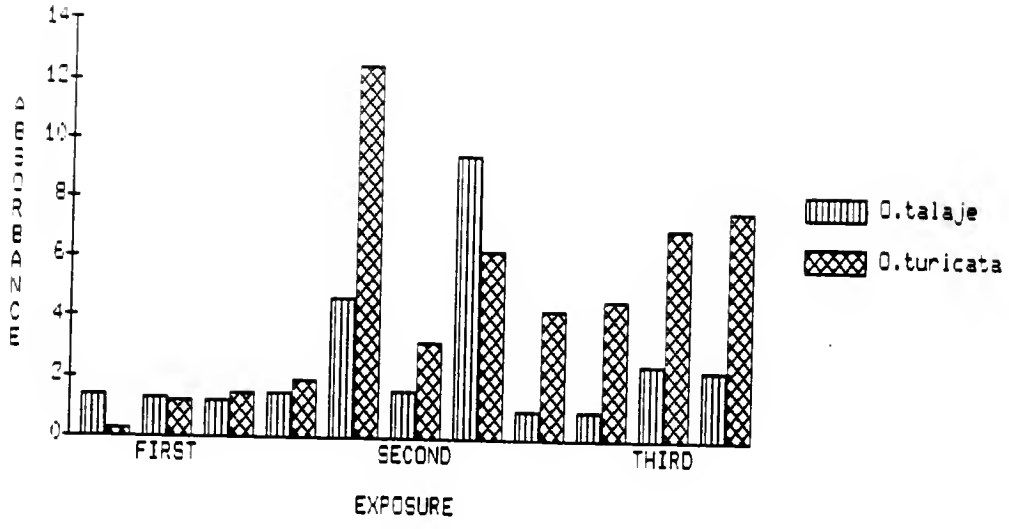

Figure 3.4. Mouse IgG1 response to three exposures of two species of Ornithodoros tick.

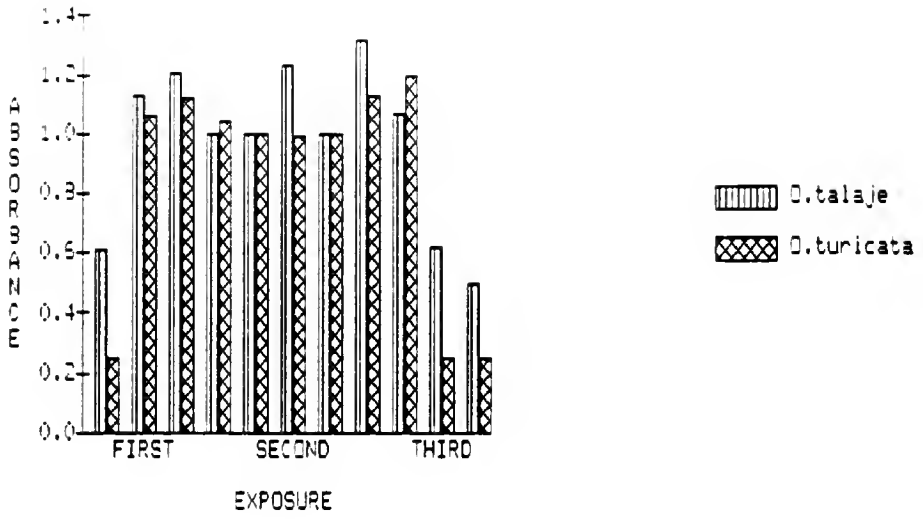

Figure 3.5. Mouse IgG2A response to three exposures of two species of Ornithodoros tick.

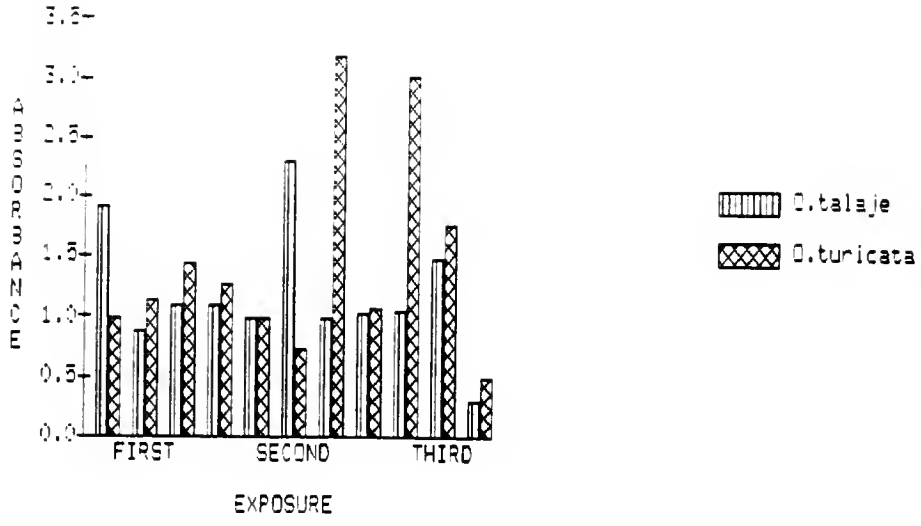

Figure 3.6. Mouse IgG2B response to three exposures of two species of Ornithodoros tick.

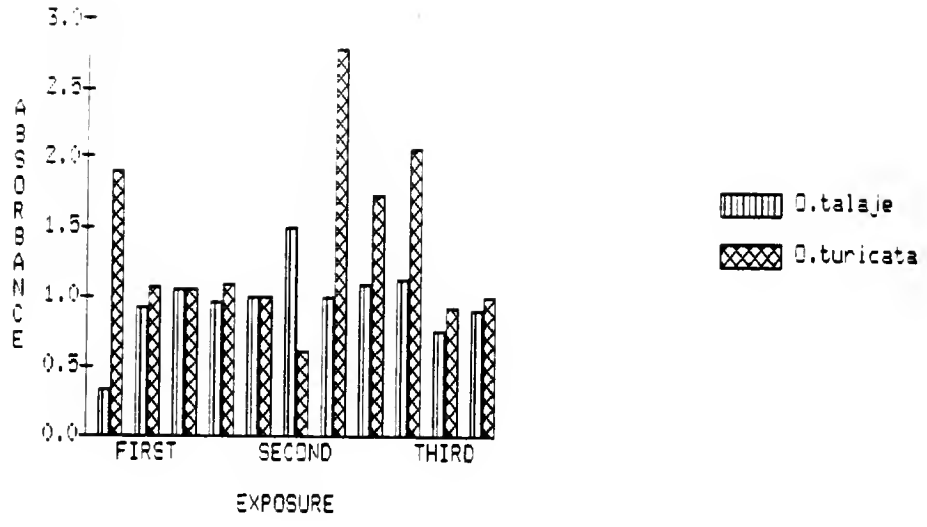

Figure 3.7. Mouse IgG3 response to three exposures of two species of Ornithodoros tick.

explained if the host antibody response to tick feeding undergoes a specific immunoglobulin class switch as it matures. Maturation of the response would mean that while increasing amounts of a more specific isotype antibody are being produced, the total volume of immunoglobulin would decrease. So, an initial rise in total Ig levels would be followed by a decrease, as the maturation process develops.

The concept of maturation of the antibody response is supported by the significant IgM and IgG1 isotypes measured after the second exposure and the fact that only a significant IgG1 isotype could be measured after the third exposure. This represents the antibody class switch mentioned above. Though an occasional individual reading for the IgG2B and IgG3 isotypes was slightly above two, it did not appear to mark a significant trend as did the IgM and IgG1 isotypes.

In one other report in which the host (hamsters) antibody response to tick feeding was isotyped, that response was determined to be of the IgG1 class (Brown and Askenase, 1983). In that case, it was reported that the mechanism for immune rejection of ticks was in part due to IgG1 antibody recruitment of effector cells. Though this mechanism has not been proven to occur in mice, mouse IgG1 antibody is reported to have both complement activating (alternative pathway) and anaphylaxis stimulating properties (Klaus et al., 1979; Mota, 1986; Spiegelberg, 1974; Voisin,

1976). These are both potentially effective mechanisms that may be responsible for the development of acquired resistance to argasid tick feeding reported in Chapter 2.

As mentioned earlier, cross-reactivity between different tick species and genera has been reported. This is not difficult to understand as many of the compounds injected during feeding are possibly similar. The cross-reactivity between argasid and ixodid ticks seen in this study, however, is unique and has not been reported elsewhere. While it may be more difficult to accept the idea that ticks of two different families and feeding strategies would use similar antigenic compounds, it becomes more believable when one considers that it has been shown that ticks and mites share antigenic proteins (denHollander and Allen, 1986).

In summary, significant antibody responses were shown to occur in mice as a result of sequential exposures to two species of argasid tick. Those responses remain detectable for at least 90 days after last exposure. Antibody isotyping has shown that the initial response is of the IgM class with a secondary class switch to the IgG1 class occurring. Finally, evidence is presented that cross-reactive proteins exist between both argasid and ixodid ticks.

CHAPTER 4
PROTEIN CHARACTERIZATION OF ARGASID TICK SALIVARY GLANDS; IDENTIFICATION OF SPECIFIC PROTEINS ELICITNG AN ANTIBODY RESPONSE

Introduction

Two basic approaches have developed utilizing the host's immune response as a means for controlling ticks (Willadsen, 1987). The first approach involves exploitation of hosts' acquired and immunologically mediated resistance after repeated tick infestations, that limits further tick feeding. In this case, the host responds to proteins injected during feeding (or to vaccinations of tick salivary gland tissue that simulate that feeding). The host-tick interface is the site of immunomodulation and the host animal basically rejects the tick. A minimum of 24-48 hours is generally required before the host response occurs, so this method may only be effective against those ticks which remain attached for several days.

The second approach involves the use of isolated tick antigens, those with which the host would not normally come in contact such as tick midgut, to induce host resistance (activate host antibody) by direct immunization. Activated antibody taken up in the bloodmeal binds to its antigenic counterpart, hopefully disrupting cellular function and

increasing tick mortality. This report deals with the first approach from the perspective of learning more about the specific proteins eliciting both an antibody response and acquired resistance in lab mice to two species of argasid tick.

As stated above, tick salivary secretions during feeding act as the inducers of the host antibody response. Several studies using polyacrylamide gel electrophoresis, host antibody, and Western blotting techniques have shown this to be true, and isolation of the specific salivary proteins responsible for host resistance to ixodid tick feeding has been conducted (Brown, 1986; Brown and Askenase, 1986a,1986b; Brown et al., 1984; Gill et al., 1986; Gorden and Allen, 1987; Shapiro et al., 1986; Whelen et al., 1986; Wikel and Whelen, 1986). Numerous antigenic proteins have been identified and considering the number of functions carried out by the salivary glands during feeding, this is not surprising.

Several studies indicate that not only do host animals express a strong acquired resistance to one species of tick, but that resistance is cross-reactive to other species and genera of ixodid ticks to which the host has not been exposed (Labarthe et al., 1985). Additional work has shown that certain proteins in mites may be cross-reactive with ticks (denHollander and Allen, 1986). This may indicate shared antigens among these hematophagous arthropods that

are important in the induction of the host antibody response and the acquisition of host resistance to tick feeding (Brown and Askenase, 1984).

To date, very little work has been done regarding the development of host resistance to argasid tick feeding. Chapter 2 presents evidence that acquired host resistance to argasid tick feeding can develop in mice and speculates that it may be antibody mediated. Chapter 3 defines that antibody response and shows that it is long lasting, of the IgG1 isotype, and appears to be cross-reactive with proteins from another argasid tick species as well as those of an ixodid tick. The purpose of this study was to identify with Western blotting techniques the salivary gland proteins responsible for induction of that host antibody response and to further define the degree of cross-reactivity.

Materials and Methods

Two species of Ornithodoros ticks were maintained in colony for use in this study. One species, O. turicata, feeds in less than one hour in all instars (Davis, 1941), typical of many argasid ticks. The other species, O. talaje, attaches for six to eight days during its larval feeding stage but feeds similarly to O. turicata at the other life stages (Cooley and Kohls, 1944). The colony ticks and laboratory mice were maintained as discussed in Chapter 2.

For this study, host exposure to the two species of tick was set up in the following manner. On three separate occasions, once every thirty days, twenty larvae of each tick species were permitted to feed on individual, 2 month old, female CD1 mice (3 mice, each tick species). The first exposure was on naive mice; the second and third exposures were to the same, now previously exposed mice. Exposure procedures are discussed in Chapter 2. Approximately 14 days after the third exposure, serum samples were obtained from each of the mice using techniques discussed in Chapter 3 and used for the Western blotting procedure.

Tick salivary glands (SG) were dissected in PBS (methods in Chapter 3) from late nymph and adult ticks of our colony O. turicata and O. talaje species, and (to check for the presence of cross-reactive salivary gland proteins) one ixodid species, Amblyomma maculatum Koch (obtained from Oklahoma State University). Thirty SG of each tick species were sonicated in 500 ul of PBS, centrifuged for two minutes (@ 3000 G), and the supernatants were stored until used for sodium dodecyl sulfate, polyacrylamide gel electrophoresis (SDS-PAGE) analysis, in separate containers at -18^{o}C. Because of the possible immunological influence of the feeding of ear mites, Psoroptes cuniculi (Delafond), on our laboratory mice, samples of whole mites were also prepared as above and run for a cross-reactivity check. Protein content of the tick samples was determined using standard assay techniques (Bradford, 1976).

SDS-PAGE was carried out in a discontinuous buffer system using a 4% 0.125M Tris buffered acrylamide stacking gel (pH 6.8) with a 14% 0.375M Tris buffered acrylamide separating gel (pH 8.8) following the system of Laemmli (1970). Samples for analysis were denatured by boiling for five minutes in 0.5M Tris-HCL bufferred 1% 2-mercapto-ethanol/10% SDS sample buffer. Low molecular weight standards (Bio-Rad Laboratories) were run in parallel for comparison and approximate molecular weight determination.

Samples of the mite (P. cuniculi) and salivary gland homogenate for each of the three tick species (O. talaje and O. turicata and A. maculatum) were run for approximately 3 hours (35 mA/gel) in .03 M Tris bufferred running buffer and used for staining with either silver stain (Merril et al., 1981) or Commassie Blue R-250 stain (Bio-Rad Laboratories), and for Western blotting (Alexander et al., 1983). Trans-blotting of the gels onto nitrocellulose membranes (Bio-Rad Laboratories) was carried out in 24mM Tris/192mM glycine/20% methanol transfer buffer using a two hour process (150 V., .55 A) (Towbin et al., 1979). The blots were stained for antigens using an immunoalkaline-phosphatase protocol (Sigma) with Ornithodoros tick species specific mouse serum (1:170 dilution) as the first antibody, alkaline-phosphatase conjugated goat anti-mouse Ig (IgG, IgM, light and heavy chain) (1:1000 dilution) as the second antibody, and using the color development reagents Nitro Blue Tetrazolium and Bromo-chloro-indoyl-phosphate. Control

mouse serum (CMS) from unexposed and non-mite infested mice from another laboratory was used (1:170 dilution) to determine background binding as well as help determine the degree of cross-reactivity among the three tick species and the one mite species.

Results

Figure 4.1 shows the proteins detected using silver stain methods from the three tick salivary gland homogenates as well as the whole mite homogenate. Though all the tick samples contained similar amounts of protein, many more protein bands were detected for the two Ornithodoros species than the Amblyomma species. Approximately 30 bands were detected for each of the argasid ticks, 20 bands for the ixodid tick and 20 bands for the mite.

Figure 4.2 is a diagramatic compilation of several separate immunoassay runs showing the tick SG antigens as well as the whole mite antigens that were recognized by the serum of mice exposed on three separate occasions to feeding by O. talaje larvae. The bands noted are ones that were not recognized by control mouse serum. The anti-talaje serum detected four O. talaje SG proteins, six O. turicata SG proteins, two A. maculatum SG proteins and four mite proteins.

Figure 4.3 is a diagramatic compilation of several separate immunoassay runs showing the tick SG and whole mite antigens recognized by the serum of mice exposed three times

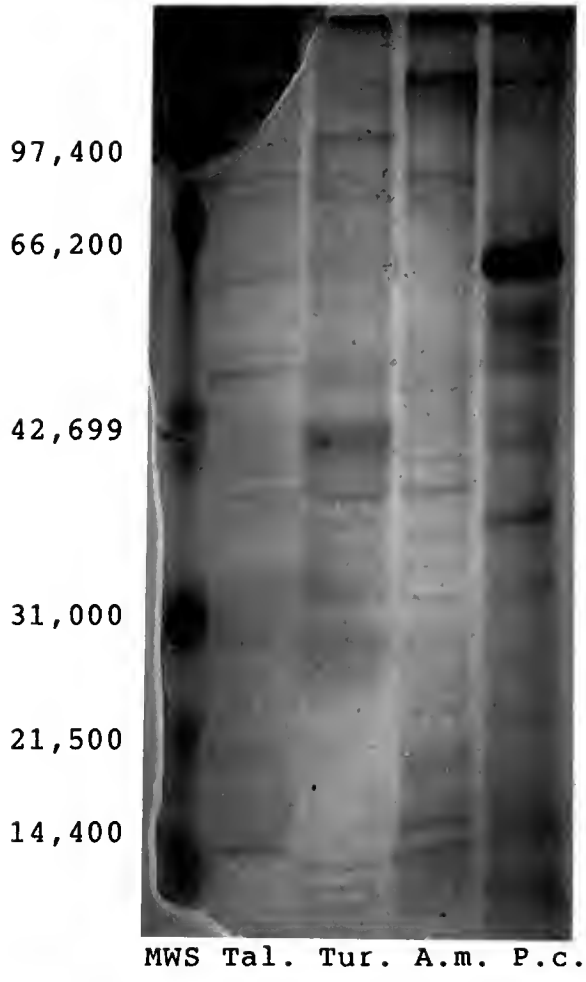

Figure 4.1. SDS-PAGE Gel for characterization of tick salivary gland and whole mite proteins, silver stained. (MWS) Molecular Weight Standards (Tal.) O. talaje, (Tur.) O. turicata, (A.m.) A. maculatum, (P.c.) P. cuniculi.

Figure 4.2. Compilation of three immunoassays showing those tick and mite proteins recognized by anti-O. talaje serum that were not recognized by control serum. (MWS) Molecular Weight Standards, (O. tal.) O. talaje, (O.tur.) O. turicata, (A.m.) A. maculatum, (P.c.) P. cuniculi.

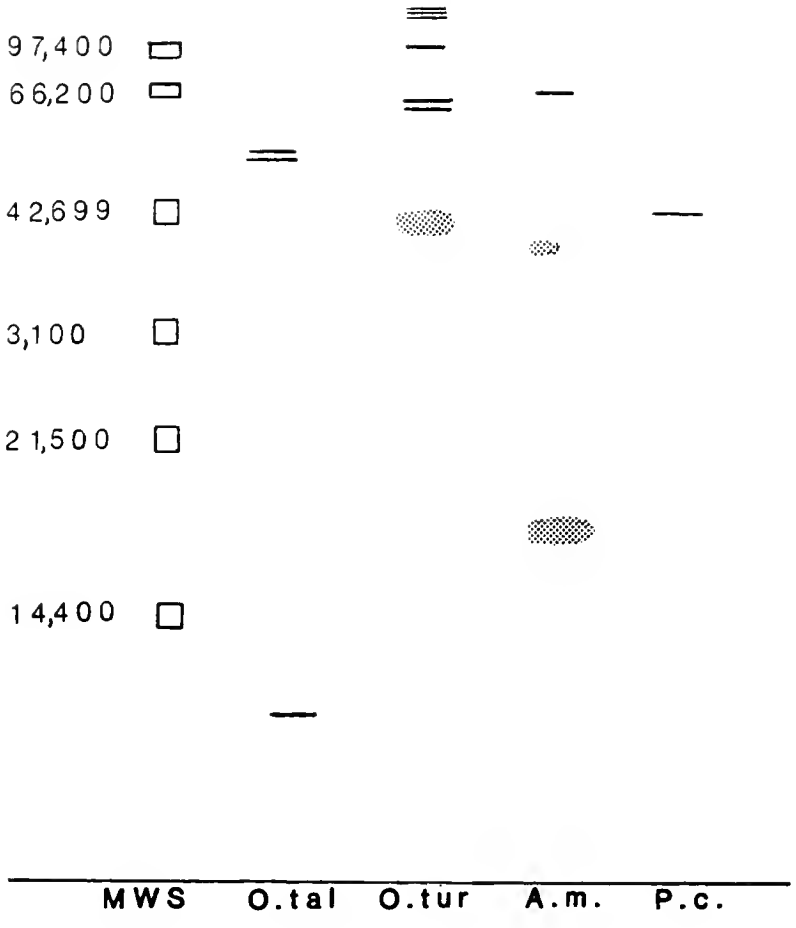

Figure 4.3. Compilation of three immunoassays showing those tick and mite proteins recognized by anti-O. turicata serum that were not recognized by control serum. (MWS) Molecular Weight Standards, (O. tal.) O. talaje, (O.tur.) O. turicata, (A.m.) A. maculatum, (P.c.) P. cuniculi.

to feeding by O. turicata larvae that were not recognized by control mouse serum. The anti-turicata serum detected three O. talaje SG proteins, seven O. turicata SG proteins, three A. maculatum SG proteins, and one mite protein.

Discussion

If the feeding stategy of argasid ticks is reviewed in regard to that of ixodid ticks, it is not surprising that a greater number of proteins were present in the Ornithodoros SG samples. Argasid ticks are typically nidiculous, feeding very rapidly on host animals straying into their burrow. This lifestyle does not permit the long term formation of various salivary proteins seen in ixodid ticks commencing upon host attachment (McSwain et al., 1982). Any and all salivary proteins required in argasid tick feeding are probably continually present, ready for utilization when the opportunity presents itself.

In examining Figures 4.2 and 4.3, antigenic proteins recognized by one of the tick species-specific serums of the same approximate molecular weight yet from the different arthropod species may represent cross-reactive proteins. Such proteins could be of great interest if that cross-reactivity could be confirmed and those proteins could be isolated for possible host immunization trials. Serum from mice exposed only to O. talaje larvae recognized two proteins (19 and 85 kd) from the argasid SGs and a third SG protein (15 kd) that was in common to both the argasid and

ixodid species. Serum from mice exposed only to the fast feeding O. turicata larvae did not recognize any cross-reactive proteins. This apparent inconsistency may be related to the behavioral feeding of O. talaje larvae which remain attached for 6-8 days permitting a more refined and specific antibody response.

Those proteins that do not appear to be cross-reactive may be species specific and would also be of interest in the development of an ELISA system that could detect what species of tick, if any, had fed on certain host animals. That information would be useful in the study of the parameters of a specific disease (J.F. Butler, pers. com.). In that case, from the results of these trials, there appear to be several proteins injected during feeding that are species specific.

Some degree of cross-reactivity does appear to exist between the argasid ticks and the ear mite. Each species-specific serum recognized one different cross-reactive protein (Fig 4.2, anti-talaje- 36kd; Fig. 4.3, anti-turicata- 42kd) that appeared to be present in both the argasid SG and the whole mite preparations. As mentioned earlier, cross-reactivity has been previously reported between mites and ixodid ticks so our findings support that data. It is quite likely that if the Amblyomma SG sample were taken from attached ticks, rather than from ones that had not been involved in feeding, more salivary proteins would have been present and cross-reactivity

between that species and the mite would also have been noted. Furthermore, if a larger amount of mite protein had been used in these experiments, more cross-reactivity between ticks and mites may have been detected.

In summary, several different cross-reactive proteins appear to be present in both argasid and ixodid tick salivary glands as well as in a Psoroptes mite. There was no protein common across the board to both the ticks and the mite, although one protein appears to be cross-reactive between tick families. Further work is necessary to confirm this first observation which shows cross-reactivity between argasid and ixodid ticks, as well as between argasid ticks and Psoroptes mites.

CHAPTER 5
HISTOLOGICAL STUDY OF THE CELLULAR RESPONSES OF LABORATORY MICE TO SEQUENTIAL FEEDINGS BY *Ornithodoros talaje* LARVAE

Introduction

In recent years, the histology of tick feeding sites has been studied in a number of tick-host systems. The results of those studies have shown that the host reaction to tick feeding is a complex phenomenon and depends to a large extent on the species of host and tick involved, the amount of time elapsed since attachment, and whether or not the host has been previously exposed. In general, the cellular reactions at tick feeding sites change in character and magnitude with time after attachment, and differ considerably from first to third infestation (Gill and Walker, 1985).

The primary infestation is typically marked by infiltration of neutrophils, followed by mononuclear cells and eosinophils. Subsequent infestations differ in that the percentage of basophils infiltrating the bite site significantly increases, causing a complementary decrease in the percentage of neutrophils and eosinophils. Often an increase in basophil and mast cell degranulation is also noted. In tick-resistant guinea pigs, for example, the

cellular responses are dominated by basophils and eosinophils (Allen, 1973; Brown and Knapp, 1981; Brown et al., 1983a; Brown and Askenase, 1984). A significant increase in basophils has also been noted in tick-resistant rabbits (Brossard and Fivaz, 1982; Gill and Walker, 1985; Walker and Fletcher, 1986), and cattle (Gill, 1986), though neutrophils still made up the greatest proportion of infiltrating cells.

The importance of basophils in the expression of host resistance to ticks was made evident when anti-basophil serum given to tick-resistant guinea pigs reduced not only the number of basophils and eosinophils, but also ablated the expression of immunity to the ticks (Brown et al., 1982). While mice may only have low levels of circulating basophils, mast-cell derived vasoamines have been shown to be involved in delayed type hypersensitivity reactions of this species (Askenase, 1977). As an example, genetically mast cell-deficient mice were unable to acquire resistance against larval ixodid ticks (Matsuda et al., 1985). When the same mice were later injected with cultured mast cells, normalization of their anti-tick response occurred (Matsuda et al., 1987). Regardless of the specific cells involved, the change in makeup of infiltrating cells as host resistance develops appears to be mediated by several immunological mechanisms, including IgG1 antibody (Brown and Askenase, 1983), complement (Wikel and Allen, 1977), and T-cell mediated responses (Brown and Askenase, 1983).

When acquired resistance to tick feeding does occur, various mechanisms may be at work. The recruitment of effector cells to the feeding site in resistant animals appears to have serious consequences for the feeding tick. The components of the host response causing these effects are not known, but possibly include degranulation products from the effector cells (Allen and Kemp, 1982; Kemp and Bourne, 1980). The most severe effect appears to be on the gut stem cells of the tick which are, for some reason, unable to proliferate or differentiate (Walker and Fletcher, 1987). It has been suggested that the resulting hypoplasia prevents the gut from accomodating a blood meal at final engorgement of equal size as that taken by ticks feeding on naive hosts.

Work done with argasid ticks has shown that guinea pigs react in much the same way to feeding by Ornithodoros ticks, (specifically O. tartakovskyi Olenev and O. parkeri Cooley). Subsequent exposures generated increases at the feeding site in both basophils and eosinophils similar to, if not greater than those seen in response to ixodid ticks (Brown et al., 1983b; Johnston and Brown, 1985). Despite mounting a strong cutaneous response of the kind that mediates immune rejection of ixodid ticks, no such effects were seen on the argasid ticks; probably a result of their fast feeding nature (McLaren et al., 1983). One exception was noted when measuring the effects of sequential feedings of Argas persicus larvae, which take at least four days for

engorgement on chickens. Partial immunity was noted and was apparently due to the longer attachment and feeding period (Trager, 1940).

Chapter 2 presents evidence that acquired host resistance to both fast and slow feeding argasid ticks can develop in mice. The purpose of this study was to observe the tick attachment site of a slow feeding larval Ornithodoros species over a series of sequential feedings to determine if histological evidence of a host immunologic reaction, possibly responsible for the acquired resistance mentioned above, could be noted. In addition, it was hoped that histological analysis of the bloodmeal taken up by the tick would provide further information about the host immunologic reaction.

Materials and Methods

The species Ornithodoros talaje is maintained in colony as described in Chapter 2, and was used in this study because it attaches for six to eight days during its larval feeding stage. A total of eight CD1 mice (female, >2 months of age) were exposed (see Chapter 2 for exposure methods) to O. talaje larvae, 20 larvae per exposure. Two of those mice were sacrificed for tissue samples at each of the following time sequences: first exposure, day 3 of tick attachment (1+3); first exposure, day 6 of tick attachment (1+6); third exposure, day 3 of tick attachment (3+3); and third exposure, day 6 of tick attachment (3+6).

The mouse tissue samples with ticks still attached were immediately placed in FAA fixative (10% formalin, 5% acetic acid, 50% ethanol, and 35% distilled water) (Carranza et al., 1987). Twenty four hours later the samples were transferred to fresh FAA and stored in stoppered vials until used (2-3 weeks) for histological preparation. The samples were then dehydrated, cleared in xylene, embedded in paraffin , cut in 4 um slices, and stained using hematoxylin and eosin following standard procedures (courtesy of the histology laboratory of the Veterans Hospital, Gainesville, FL). Photomicrographs were taken using a Zeiss Photomicroscope III.

Results

Figures 5.1- 5.4 show the larval tick attachment site for the following exposure sequences; A) 1+3, B) 1+6, C) 3+3, and D) 3+6. Figures 5.5-5.8 more closely show the mouse tissue at the tick attachment site for the above sequences, permitting one to concentrate on the specific host cells trafficking into the bite site. Figures 5.9 and 5.10 are presented to show the change in tick morphology that occurs between day 3 and day 6 of feeding. Figures 5.11-5.14 are close up views of the contents of the tick gut for the above host sequences showing the specific host cells that have been taken up and concentrated in the tick gut during feeding.

Examination of the feeding site shows that attachment is to the epidermis of the mouse only, the mouthparts of the tick never penetrating beneath that layer (Figs. 5.1, 5.3, and 5.4). While epidermal penetration does not occur, it is quite obvious that tick attachment causes a significant host reaction to occur in the dermis beneath that site (Figs. 5.2, 5.3., and 5.4).

The sequence of events is as follows. First exposure, day 3, (Fig. 5.1) a loosening of collagen resulting from dermal edema and a very mild inflammation of the dermis is present. No unusual concentration of host cells appears to be present beneath the attachment site (Fig. 5.5). First exposure, day 6, (Fig. 5.2) more intense inflammation of the dermis is noted and a foci of polymorphonuclear cells, mostly neutrophils (10:1) is present. A few individual lymphocytes and macrophages are also present (Fig 5.6). Third exposure, day 3, (Fig 5.3) a much more intense inflammation of the dermis is noted (more so than either the 1+3 or the 1+6 exposures) and while a large number of neutrophils are present, comparatively more lymphocytes (more nearly 1:1) are present (Fig. 5.7) than in 1+6. Third exposure, day 6, (Fig 5.4) though the epidermis has not been penetrated, it does appear to be broken and host cells appear to be actively moving into the newly formed epidermal feeding cavity. Some epidermal necrosis can also be noted. The level of dermal inflammation is more intense than that of 3+3 and while many neutrophils are present (many appear

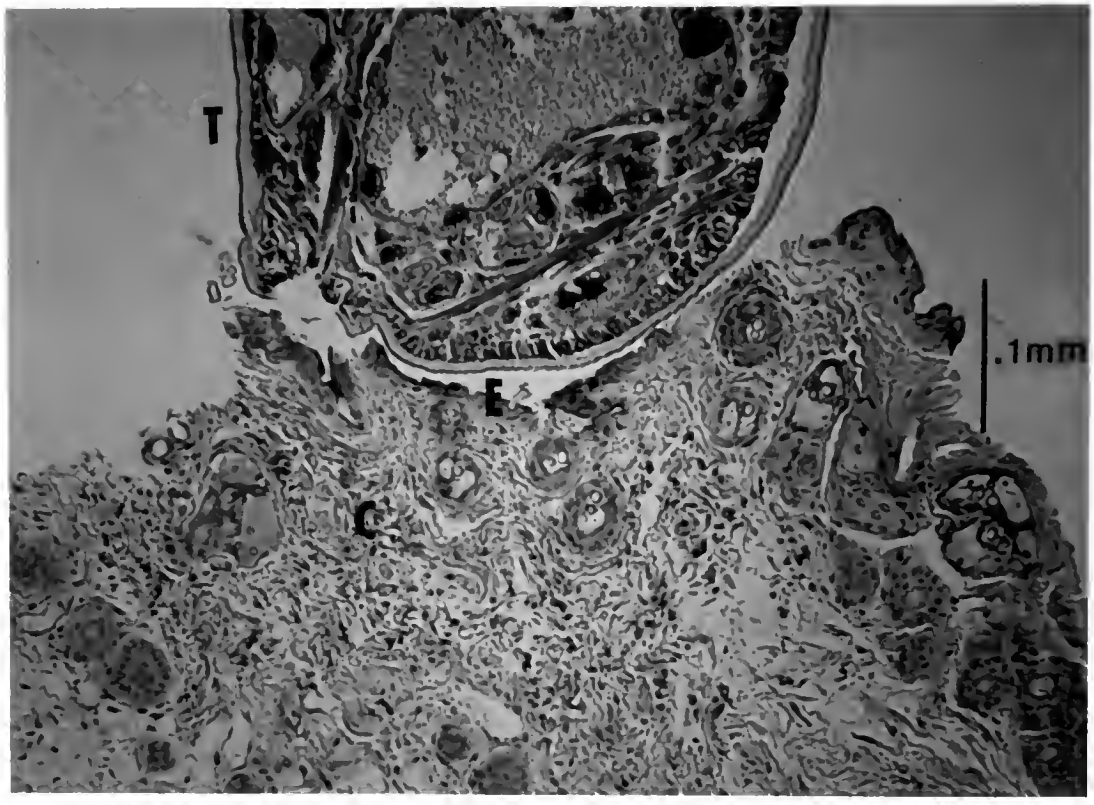

Figure 5.1. Tick attachment site; first host exposure, day 3. (E) Epidermis, (C) Collagen, (T) Tick.

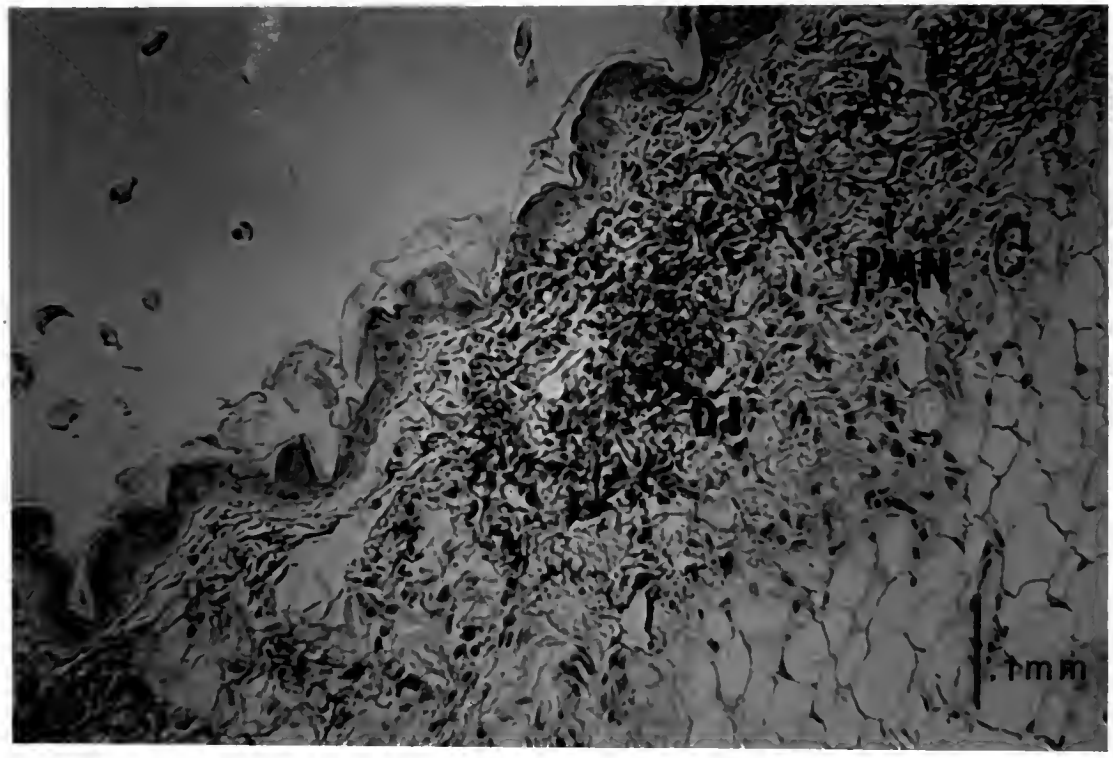

Figure 5.2. Tick attachment site; first host exposure, day 6. (DI) Dermal Inflammation (PMN) Polymorphonuclear cells.

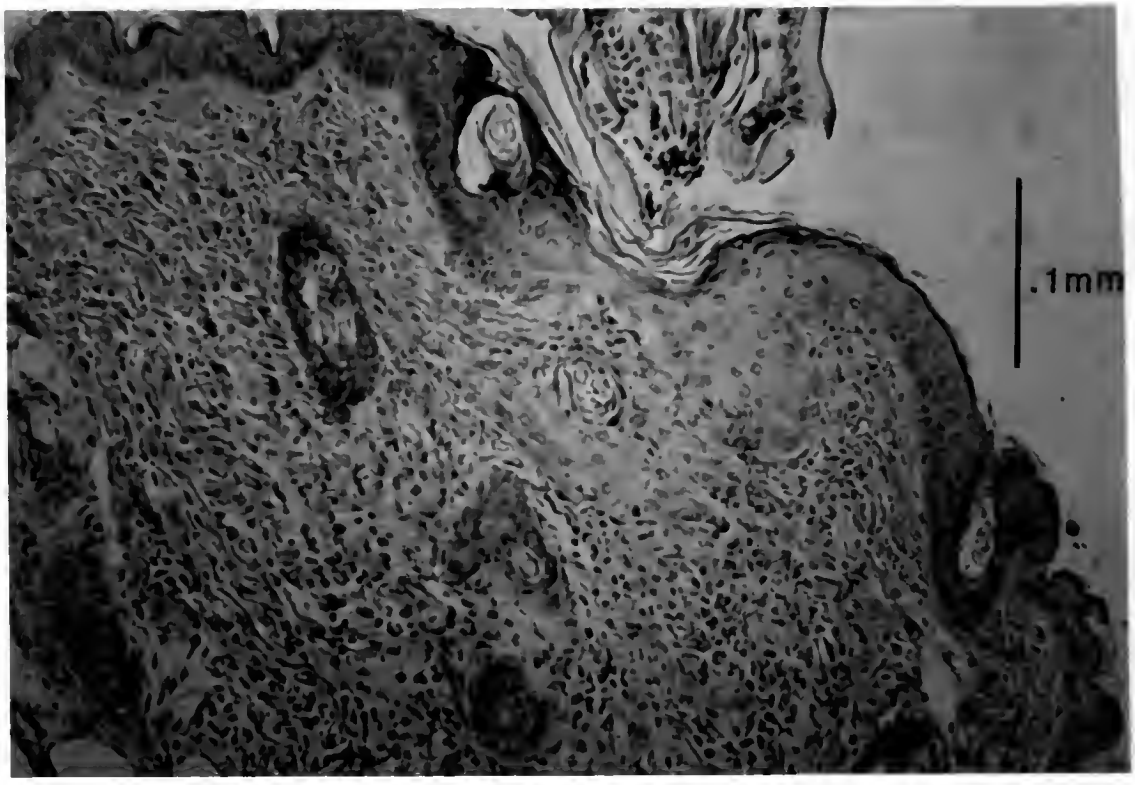

Figure 5.3. Tick attachment site; third host exposure, day 3.

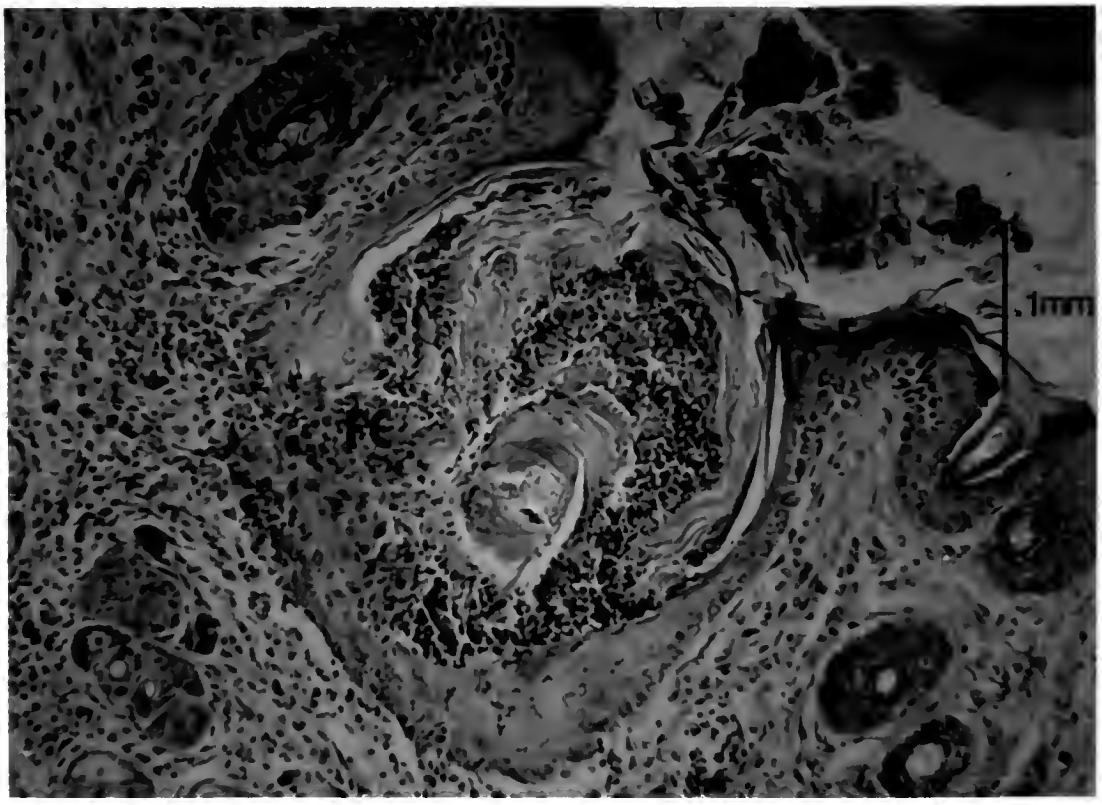

Figure 5.4. Tick attachment site; third host exposure, day 6. (E) Epidermis, (FC) Feeding Cavity.

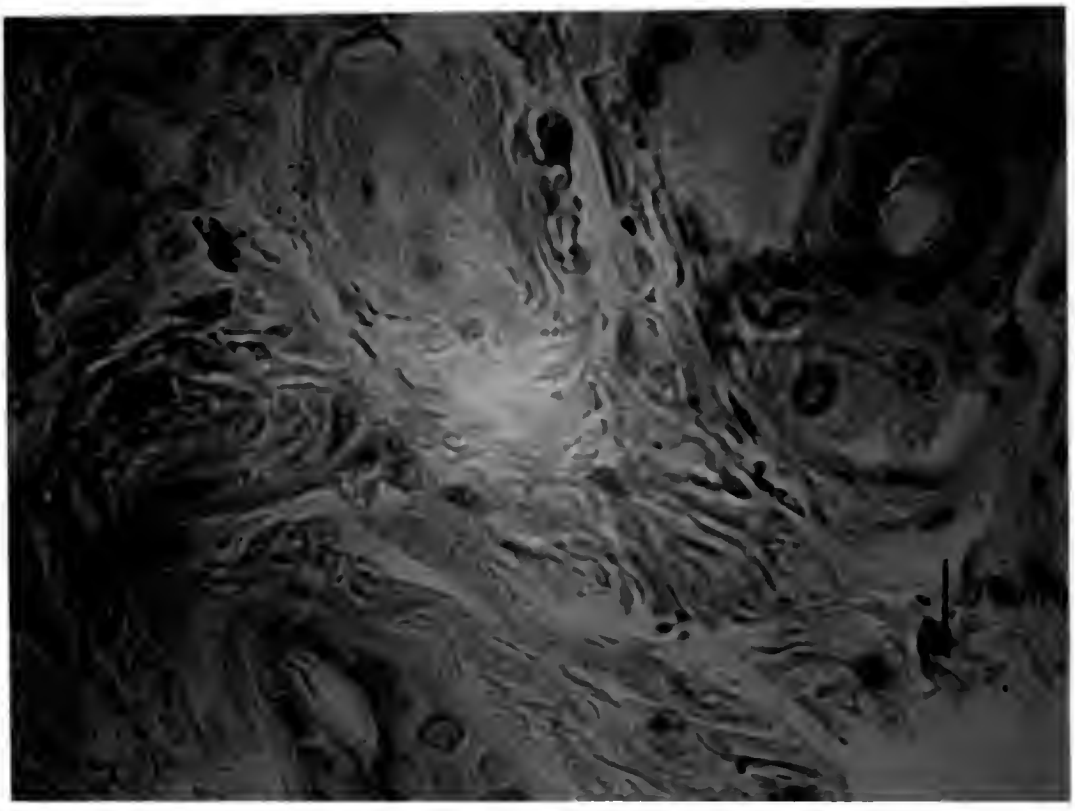

Figure 5.5. Tick attachment site; first host exposure, day 3.

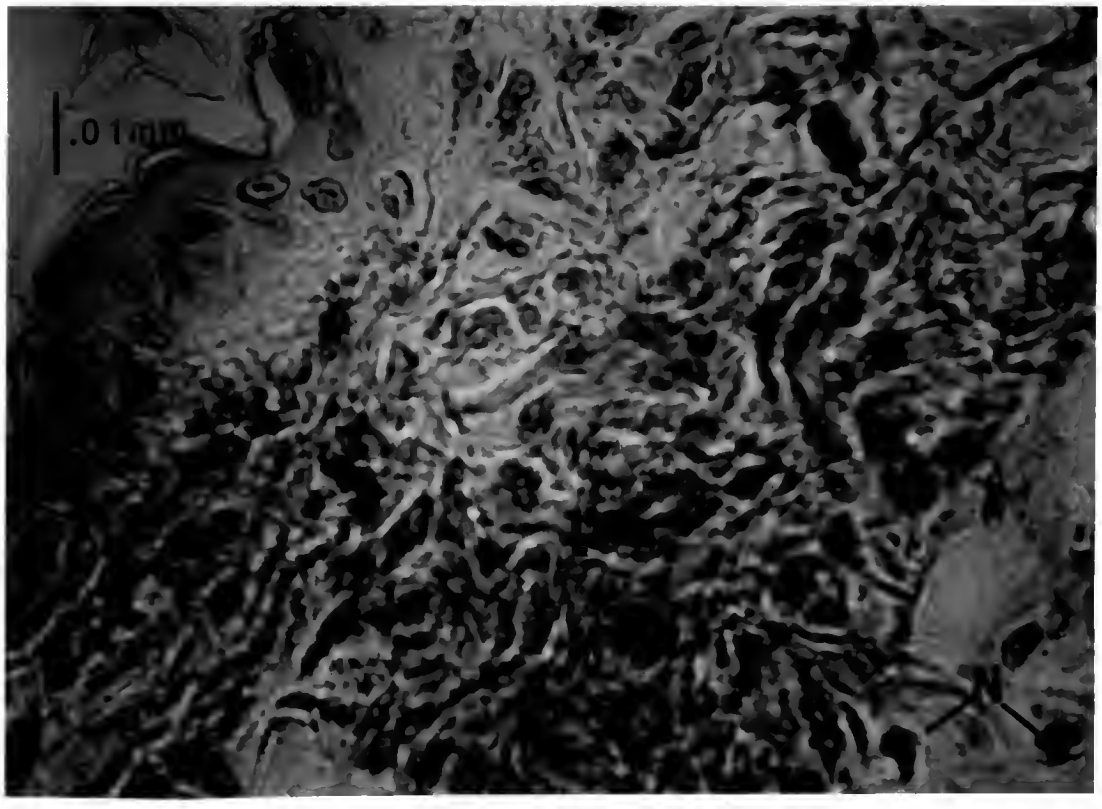

Figure 5.6. Mouse tissue at the tick attachment site; first exposure, day 6. (L) Lymphocyte, (M) Macrophage, (N) Neutrophil.

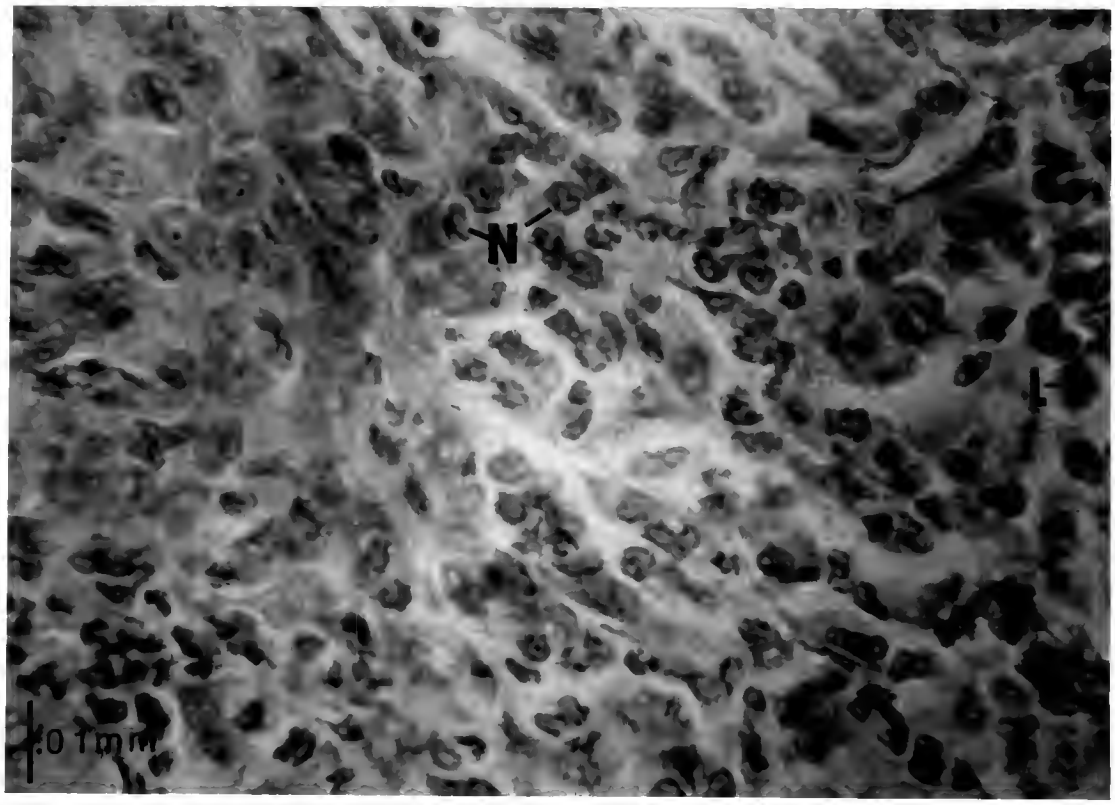

Figure 5.7. Mouse tissue at the tick attachment site; third exposure, day 3. (L) Lymphocyte, (N) Neutrophil.

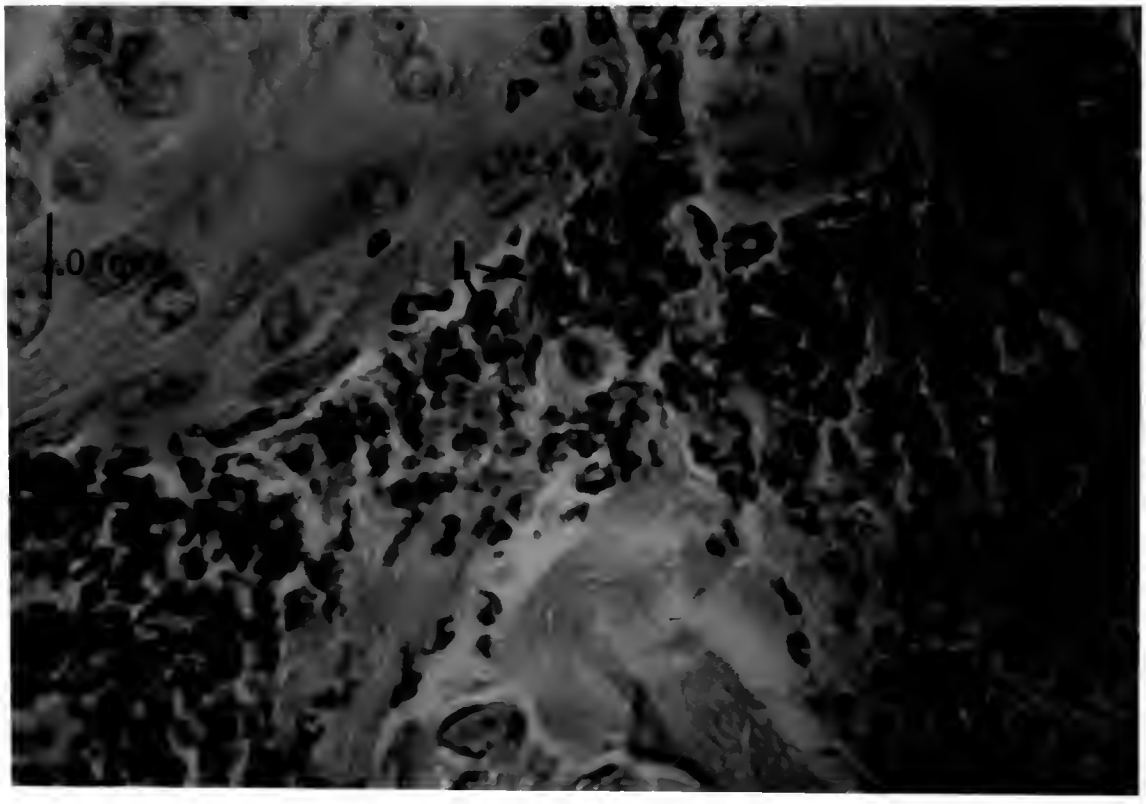

Figure 5.8. Mouse tissue at the tick attachment site; third exposure, day 6. (L) Lymphocytes.

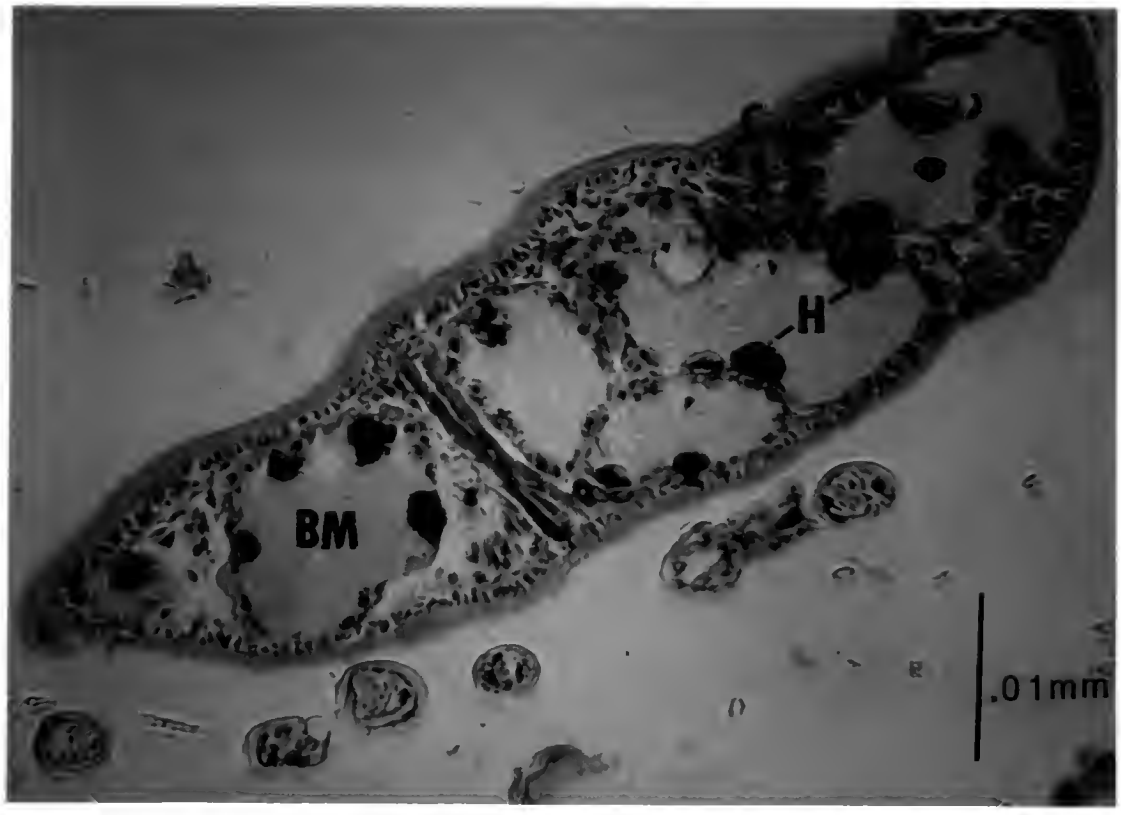

Figure 5.9. Longitudinal section of attached tick; first host exposure, day 3. (BM) Blood Meal, (C) Cuticle, (H) Hematin.

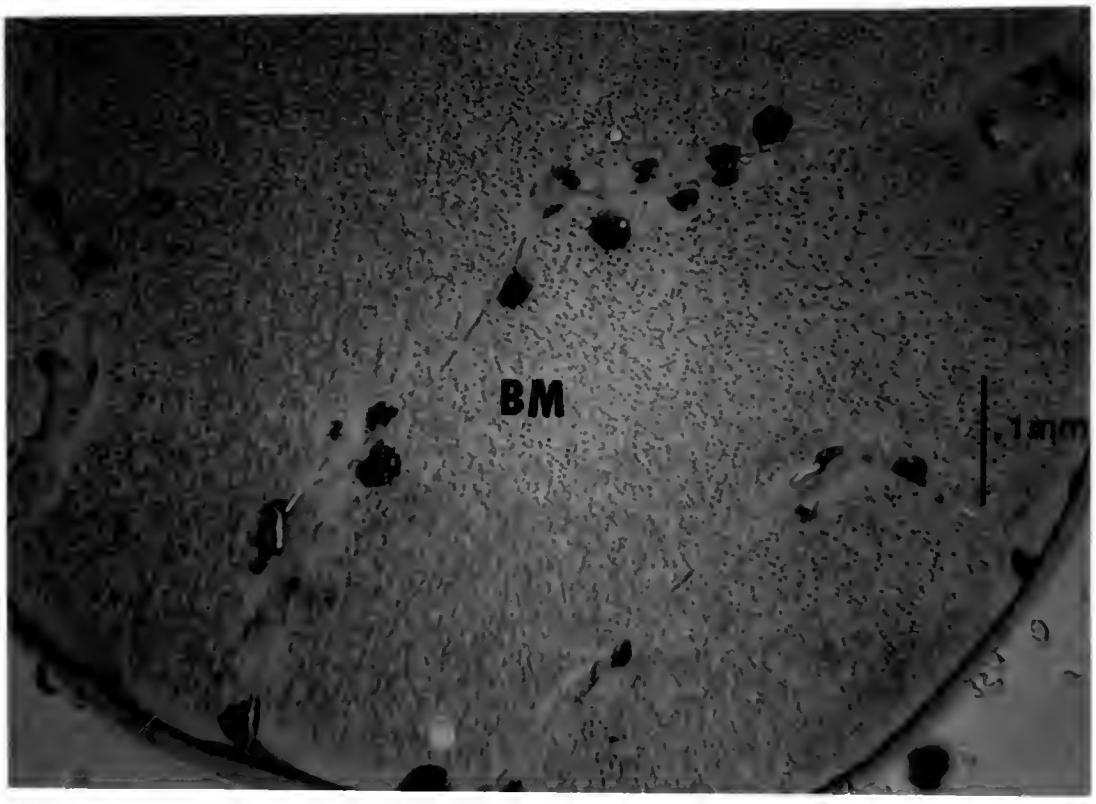

Figure 5.10. Cross-section of attached tick; first host exposure, day 6. (BM) Blood Meal, (C) Cuticle.

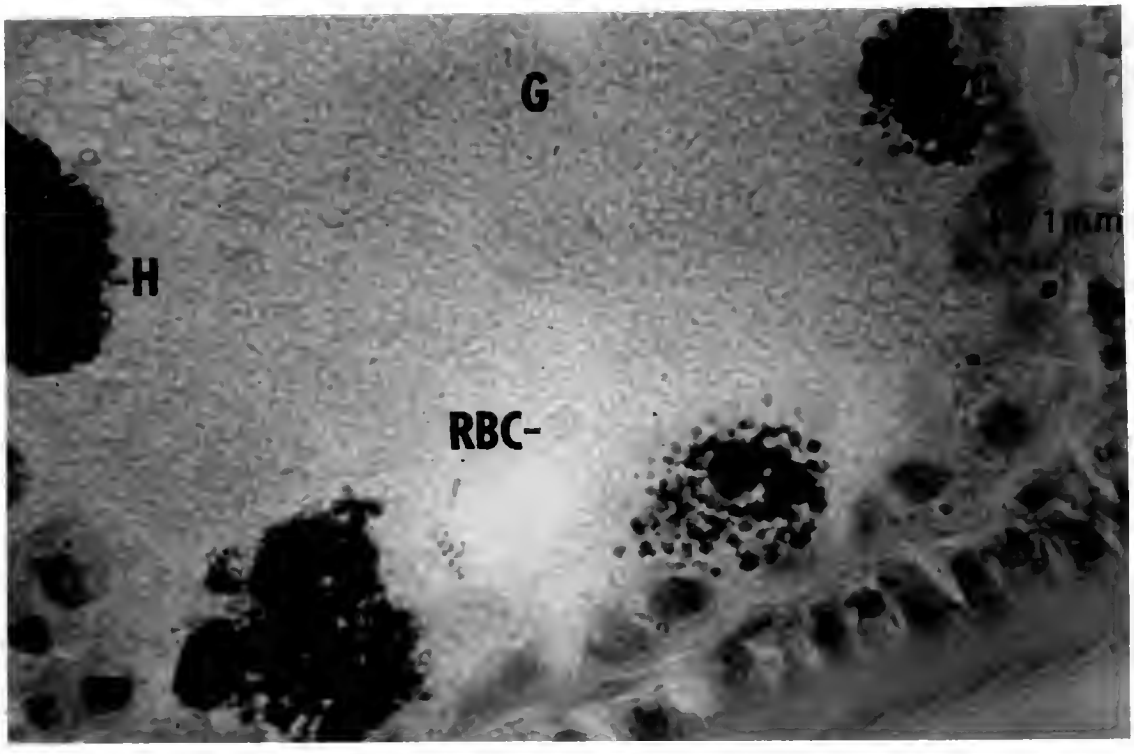

Figure 5.11. Tick gut; first host exposure, day 3. (RBC) Red Blood Cell, (C) Cuticle, (G) Gut, (H) Hematin.

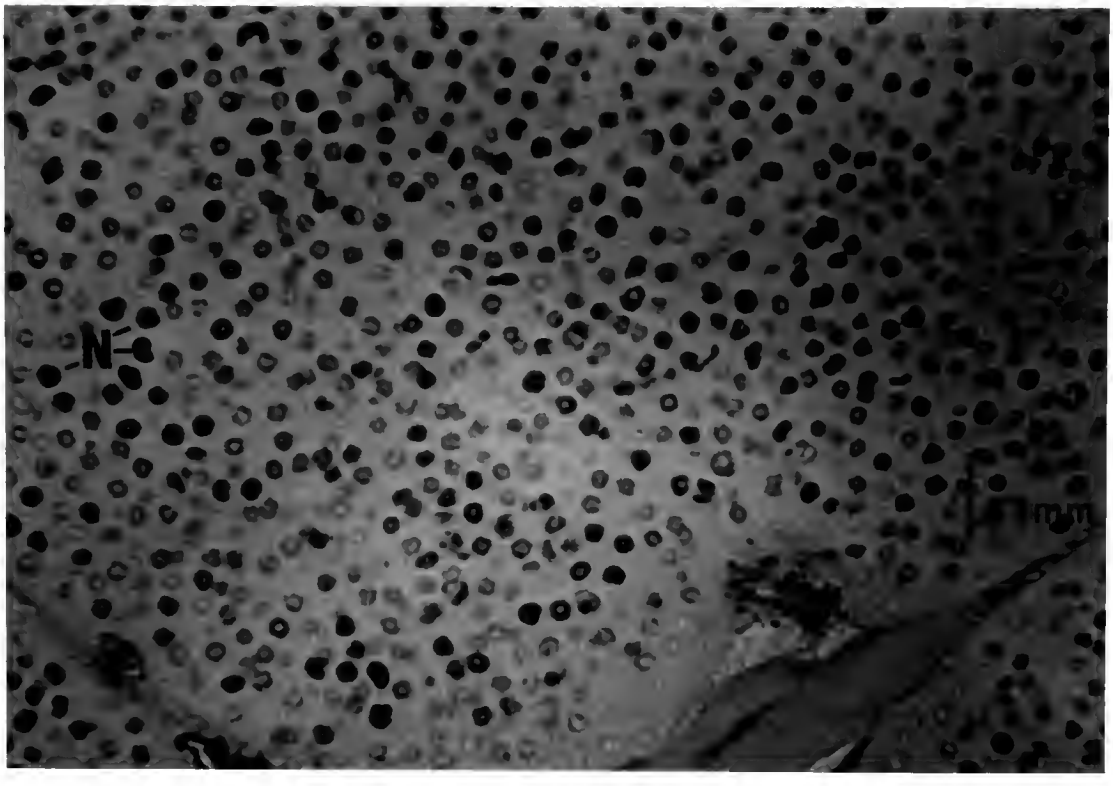

Figure 5.12. Tick gut; first host exposure, day 6. (N) Neutrophils.

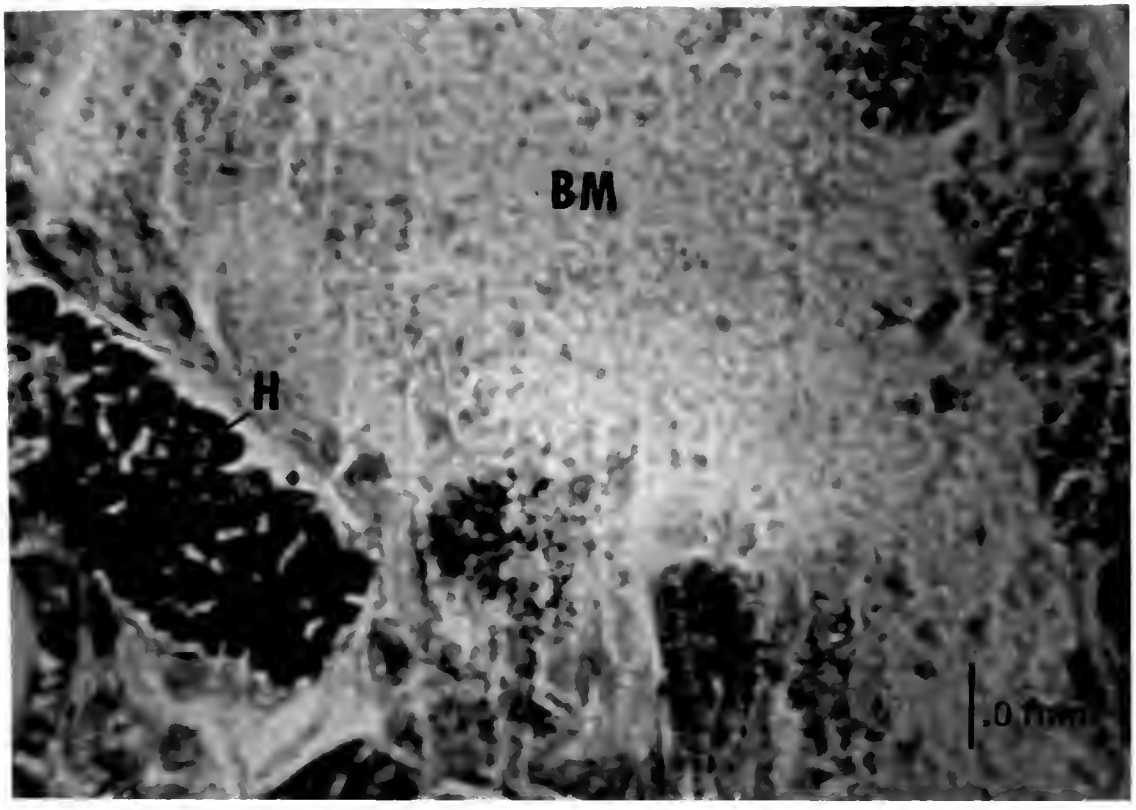

Figure 5.13. Tick gut; third host exposure, day 3. (H) Hematin, (BM) Blood Meal.

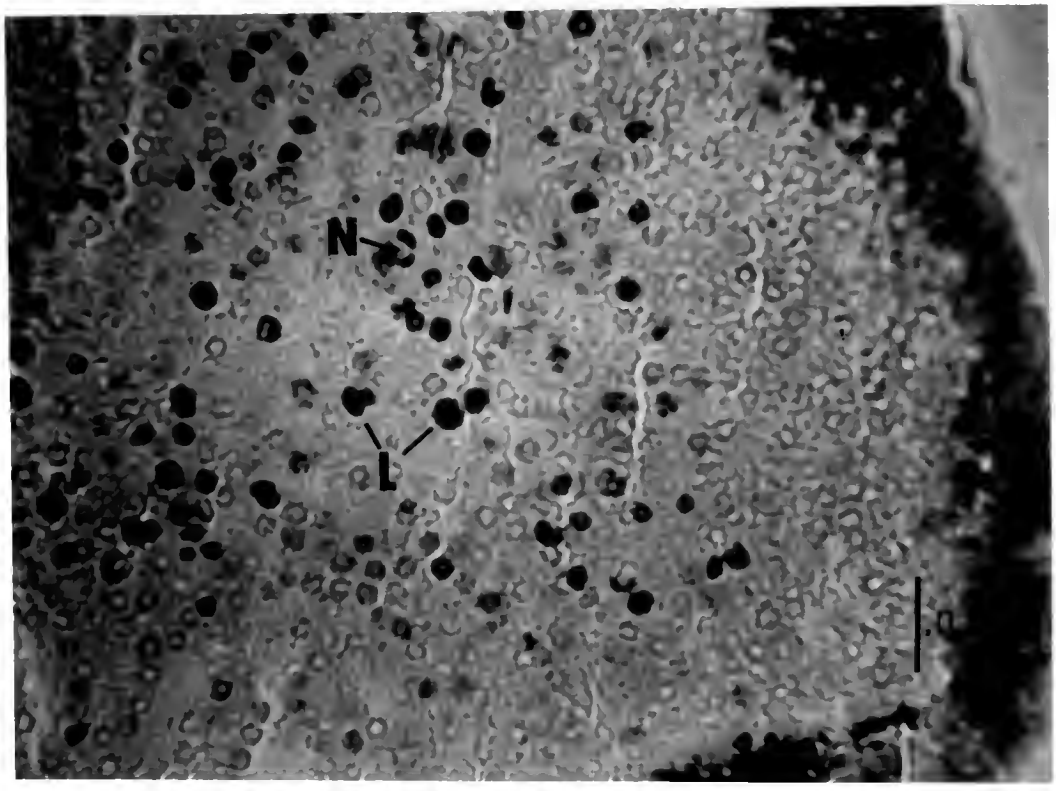

Figure 5.14. Tick gut; third host exposure, day 6. (L) Lymphocyte, (N) Neutrophil.

degenerative) a definite shift to lymphocytes is noted (Fig 5.8). An occasional mast cell (not shown) was also noted on the periphery of the feeding cavity and it appears that more mast cells were present than at any other time.

Histological examination of the host cells taken up by the tick during feeding is also very informative. First exposure, day 3, (Fig 5.11) no host cells are apparent in the tick gut, though an occasional ghost of a red blood cell can be distinguished. Hematin granules (the remains of hemoglobin digestion) are present showing that host blood digestion is already underway. First exposure, day 6, (Fig. 5.12) numerous host leukocytes, mostly neutrophils, are present in the gut contents. Third exposure, day 3, (Fig. 1.3) much like day 3 of the first exposure, no host cells can be noted in the the gut though hematin is present. Third exposure, day 6, (Fig 5.14) again host leukocytes are present but many, if not most of those cells appear to be larger, non-polymorphic, and lymphocyte-like.

Discussion

Evidence abounds that an amnestic response is occurring in the mice to sequential feedings by the larval argasid ticks. If one looks at the mouse tissue, a significant infiltration of neutrophils into the dermis beneath the bite site occurs between day 3 and day 6 of the first exposure. On day 3 of the third exposure however, an even more intense response is noted than at day 6 of the the first exposure,

made up of an apparently equal number of neutrophils and lymphocytes. Finally, between day 3 and day 6 of the third exposure, a shift from mostly neutrophils to mostly lymphocytes occurs.

If the tick gut contents are considered, the above shift from neutrophils in the first exposure to lymphocytes in the third exposure is again supported. It is interesting to note that no host cells were evident in the gut contents of day three ticks regardless of whether they were feeding on naive or previously exposed hosts. This could reflect the fact that the ticks may not begin to feed in earnest for the first few days of attachment but may also be artifactual due to the fact that a cross sectional view of the 1+3 and 3+3 ticks was not made.

The transition or shift in the type of host cells becoming involved at the tick attachment site during the sequential feedings is evidence that the host immune system has recognized that tick feeding is occurring and is capable of responding very quickly to that feeding the third time it occurs. In the first exposure we see an increase in neutrophils over the six days of attachment. Neutrophils are somewhat effective defensive cells, but not antigen specific. In the third exposure, an immediate lymphocyte response is seen, probably antigen specific T-cells, typical of a delayed hypersensitivity reaction. This very probably results in the development of acquired host resistance noted

in Chapter 2, causing decreased % survival of attached O. talaje larvae on previously exposed mice.

Typically, eosinophils play an important part in the sequence of a host response to parasite infestation. They do not appear to be involved in any significant manner in the immune response described here, though that may be partially a result of the fact that the tissue samples were not differentially stained to show either eosinophils or basophils. Though differential staining was not carried out, occasional mast cells/basophils did occur and were more evident beneath the attachment site of the third exposure. Without differential staining and more thorough studies, it is difficult to say anything definitive about either antibody-mediated immediate hypersensitivity or antibody-dependent cell mediated cytotoxicity.

In summary, a sequential histological study of the attachment site of a long feeding argasid tick has shown that a definite host immune response occurs in CD1 mice. While a strong neutrophil response occurs during the first exposure, the third exposure is marked by a shift to lymphocytes. Not only is this reflected in the mouse tissue but also in the cells found in histological preparations of the feeding ticks. This evidence of a lymphocytic response is probably closely related to the development of acquired resistance in mice to sequential tick exposure discussed in Chapter 2.

CHAPTER 6
LYMPHOCYTE PROLIFERATION TEST: FURTHER STUDY OF THE CELLULAR RESPONSE OF LABORATORY MICE TO SEQUENTIAL FEEDINGS BY *Ornithodoros talaje* LARVAE

Introduction

While T-helper cells initiate the humoral response, they are also involved in the series of events known as the cell-mediated immune response. Once stimulated by soluble factors released by phagocytosis, the T-helper cells begin secretion of T cell growth factors on their own such as interleukin-2. Under its influence T cells that are in contact with antigen begin proliferation, forming large clones of T cells capable of recognizing that antigen. Three T cell types proliferate: helper T cells, cytotoxic T cells, and suppressor T cells, each with it own function. These cells interact to assist the antibody response, traffic throughout the body to recognize and attack foreign antigen through cell membrane disruption, and then slow down and check the immune response once the antigen has been eliminated (Raven and Johnson, 1987). These responses together are referred to as delayed hypersensitivity reactions.

Direct evidence showing the involvement of T cells in the expression of host resistance to tick feeding is

minimal. Acquired resistance has been transferred from resistant donors to naive recipients with viable lymph node cells or peritoneal exudate cells (Askenase et al., 1982; Brown and Askenase, 1981), indicating T cell mediated involvement. In vitro work with lymph node proliferation responses to ixodid tick salivary antigens from guinea pigs undergoing first and second infestations also supports the idea that T cells are involved in the development of acquired host resistance (Wikel et al., 1978). Antigen specific in vitro blastogenesis of lymphocytes derived from first exposure animals was detected 2-4 days after termination of the first infestation. Peak responsiveness occurred 24 hours after the second infestation, falling off from that point on (Wikel and Allen, 1982). The authors suggest that the decrease in lymphocyte activity observed after the first infestation might be a result of tick induced immunosuppression.

While no published work has been noted regarding host T cell responses to argasid tick feeding, histological work discussed in Chapter 5 describes a shift from neutrophils at the tick attachment site in the first host exposure to lymphocytes at the tick attachment site in the third host exposure, indicating a T cell response. The purpose of this study was to conduct a test of mouse lymphocyte responsiveness to sequential feedings of O. talaje larvae to further define that response.

Materials and Methods

To determine if argasid tick feeding stimulates a T cell response in mice, an *in vitro* test measuring antigen-induced T cell proliferation in response to tick salivary gland antigen was conducted. This can be measured by noting if an increase in tritium-labelled thymidine uptake by previously tick-exposed mouse T cells occurs upon presentation of tick salivary gland antigen. Increased uptake indicates antigen recognition and that lymphocyte proliferation has occurred. The species *Ornithodoros talaje* is maintained in colony as described in Chapter 2, and was used for this study because work discussed in Chapter 5 showed that a significant cellular response occurred in CD1 mice resulting from sequential tick exposures.

Salivary glands from *O. talaje* ticks were dissected in phosphate buffered saline (PBS), sonicated, centrifuged, and the supernatants were frozen at -18^{o} C until used as antigen (see Chapter 3 for details). Protein content was determined using standard techniques (Bradford, 1976). T cells were obtained from cervical and axial lymph nodes removed from CD1 mice ten days after first being exposed to 20 larvae (1+10), three days after infestation for the third time (3+3), as well as from naive mice. Three mice were utilized for each exposure sequence. Suspensions of lymph cells from the three mice were prepared by gently forcing the nodes through fine mesh screens in PBS and the number of cells were standardized using a hemocytometer. Most of the cells

obtained from the lymph nodes are T cells. The assays for the naive and 1+10 exposure mice were replicated three times, the assay for the 3+3 exposure mice was not replicated. All assays were carried out in flat bottomed microtiter plates (No. 3596, Costar, Boston, MA). Ten ug per well of salivary gland antigen were added to each of the cultures and the cultures were harvested on day 4 after a 12 hour pulse with 2 uCi tritiated thymidine (Shaut et al., 1984). Thymidine uptake was measured using a Beckman brand scintillation counter (Model LS 7500).

Results

Table 6.1 presents the results of the lymphocyte proliferation test. The ratio of W/WO (counts per minute for lymphocytes presented with antigen/ counts per minute for lymphocytes not presented with antigen) is a measure of the degree of lymphocyte activity. Values >1 signify that the presence of the antigen has stimulated activity. The mean W/WO values for the naive, 1+10, and 3+3 mice were 0.8, 1.8, and 0.09, respectively.

Discussion

The lymphocyte activity (nearly 1) for the naive mice upon exposure to tick antigen was as expected, considering it was the first time those cells had been exposed to components of tick salivary gland. The level of activity found in the cells 10 days after the first host exposure to

Table 6.1. Results of lymphocyte proliferation tests using naive, first exposure, and third exposure CD1 mice.

Exposure	Thymidine Uptake Mean CPM		W/WO (mean)
	WO/Ag (SD)*	W/Ag (SD)	
Naive	427 (165)	386 (83)	.90
	786 (169)	533 (255)	.68 (.8)
	3739 (1145)	3262 (575)	.87
1+10	1115 (179)	2231 (132)	2.00
	428 (68)	984 (708)	2.30 (1.8)
	335 (80)	353 (132)	1.10
3+3**	1816	277	.15
	3841	270	.07 (.09)
	3417	142	.04

* Standard deviation
** Not replicated

ticks was greater than 1, showing that recognition of antigen occurred, and proliferation of tick antigen recognizing T cells was occurring. This level of activity is very much like that reported previously. The very significant lack of activity found in the cells 3 days after the third infestation is however the most interesting and difficult to explain.

As mentioned earlier, this same depression in activity was reported after a second tick exposure and the authors suggested that some type of immunosuppression mediated by the ticks themselves might be at work. Assuming such immunosuppression was tick mediated, this does not explain then why the host animals reacted in a positive manner to the first exposure. Two alternative mechanisms should be considered.

The first possibility is that upon antigen stimulation following the third exposure, the T suppressor cells have been activated, suppressing both T helper and T cytotoxic cell activity. While this is a reasonable explanation, it is not supported by the increased lymphocyte activity found at the tick attachment site day 3 of third exposure, discussed in Chapter 5.

The second possibility is that an event referred to as "homing" occurred (A.B. Peck, pers. com.). Hours after the ticks became attached for the third time, salivary antigens were secreted, trafficked through the lymph nodes, and stimulated a large scale movement of tick antigen

recognizing T cells from the lymph nodes to the site of attachment. A temporary lack of those cells at the time of lymph node removal would explain the apparent suppression of lymphocyte activity seen here and reported previously (Dietsch et al., 1985; Trotter and Steinman, 1984). This possibility is supported by the histological evidence mentioned above.

Accepting both the second hypothesis and the histological evidence of Chapter 5, it appears that a strong T cell response is stimulated by sequential feedings of larvae of the long feeding argasid tick O. talaje. That T cell response is very likely responsible for the development of acquired host resistance discussed in Chapter 2.

CHAPTER 7
POSSIBLE APPLICATIONS OF THE IMMUNE RESPONSE OF LABORATORY MICE TO THE FEEDING OF ARGASID TICKS

Introduction

As mentioned in Chapter 4, two basic approaches have developed utilizing the host's immune response as a means of controlling ticks (Willadsen, 1987). The first approach involves exploitation of the fact that hosts acquire an immunologically mediated resistance after repeated tick infestations that limits further feeding. In this case, the host responds to proteins injected during feeding or to vaccinations of tick salivary glands. The host-tick interface is the site of immunomodulation and the host animal basically rejects the tick. A minimum of 24-48 hours is generally required before the host response occurs, so this method will only be effective against those ticks that remain attached for several days.

The second approach involves the use of isolated tick antigens that the host would not normally come into contact with such as tick midgut, to induce host resistance (activate host antibody) by direct immunization. Activated antibody taken up with the bloodmeal binds to its antigenic counterpart within the tick, possibly disrupting cellular function and increasing tick mortality. This study deals

with this approach from the perspective of possibly developing methodology for immunologically controlling fast feeding argasid ticks.

This approach was first shown to be of great potential as a means of mosquito control in 1972 (Algar and Cabrera, 1972). Anopheles stephensi mosquitoes that fed on mosquito midgut immunized host animals showed a significantly higher mortality rate compared to mosquitoes feeding on control animals. Similar results were found with Stomyxs calcitrans (Schlein and Lewis, 1976). It was first proposed as a concept for tick control in 1975, (Galun, 1975) and successfully carried out in 1979 (Allen and Humphreys, 1979). Ticks fed on guinea pigs that had been immunized with tick internal organs suffered drastic reduction in engorgement weight and a significant reduction in egg laying. That finding led to the discovery that host antibody, taken in a bloodmeal, could move across the gut lining and enter the hemolymph of the tick (Ackerman et al., 1981). Since that time, artificially induced, partial immunity has been shown in three host species against five ixodid (Willadsen, 1987), and one argasid tick species (Chinzel and Minoura, 1988). Most recently, antigenic material has been isolated from semi-engorged Boophilus microplus ticks that produces an immunity different from and more effective than naturally acquired immunity (Willadsen et al., 1988).

With the exception of the one paper mentioned above, all of the work done thus far has been directed at controlling long feeding ixodid ticks. The purpose of this study was to conduct some preliminary investigations into utilizing this concept for the control of fast feeding argasid ticks. If successful, it would give a clear indication that the immunization process was successful at controlling argasid ticks through uptake of activated antibody and not related to a simple acceleration of the process of naturally acquired immunity.

Materials and Methods

Two species of Ornithodoros ticks were maintained in colony for use in this study. One species, O. turicata, feeds in less than one hour in all instars (Davis, 1941). The other species, O. talaje, attaches for six to eight days during its larval feeding stage but feeds similarly to O. turicata at the other life stages (Cooley and Kohls, 1944). The colony ticks and laboratory mice were maintained as discussed in Chapter 2.

The prospective antigens used for immunization, tick gut and other internal organs, were dissected, sonicated, and centrifuged following the same procedures (Chapter 3) used for preparing tick salivary glands for antigen usage. Protein determinations were made using standard assay techniques (Bradford, 1976). For each antigen used, three CD1 mice (female, >2 month old) were immunized on

three separate occasions (subcutaneously, in each hind leg and in the scruff of the neck) using a total of 100ug of the tick antigens, following the immunization schedule and using the adjuvents listed in Table 7-1.

Approximately two weeks after the third immunization, 20 ticks of the specified stage and species were then allowed to feed on each of the immunized hosts (60 ticks per treatment) and held for determination of survival. Survival of ticks on immunized hosts was compared to that of ticks feeding on both naive and control immunized hosts. Chi square analysis of survival for each treatment was conducted.

Host antibody response (1:50 dilution) to the immunization was confirmed prior to each feeding trial using either a radioimmunoassay (Hood et al., 1984) or an ELISA test (Voller et al., 1980) and the corresponding antigen. Antibody isotyping of the immunized mice was conducted in immunization experiments G,H, and K, to determine the class of antibody stimulated by immunization (see Chapter 3 for methods). To confirm that mouse antibody was crossing the tick gut in the case of the internal organ immunization series, both hemolymph and coxal gland fluid were taken from O. turicata ticks that had just completed feeding on an immunized and isotyped mouse as well as a non-immunized control mouse (Vaughan and Azad, 1988) and analyzed (ELISA) for the presence of isotype specific anti-tick antibody.

Table 7.1. Immunization procedures, antibody titre, and tick survival following immunization of CD1 mice as a possible control mechanism for argasid tick control.

Immunization		Adjuvent	Antibody Titre	Challenged with:	Per cent Survival
A)	None	--	--	O.talaje larvae	78
B)	Control Immunized	CFA, IFA (2xs) every 14 days	--	O.talaje larvae	75
C)	O. talaje salivary gland	CFA, IFA (2xs) every 14 days	15xs	O. talaje larvae	63
D)	O. talaje gut	CFA, IFA (2xs) every 14 days	18xs	O. talaje larvae	53
E)	O. talaje gut	CFA, IFA (2xs) every 7 days	13xs	O. talaje larvae	66
F)	O. talaje gut	RIBA, every 14 days	54xs	O. talaje larvae	65
G)	O. talaje gut	IFA 3xs	12xs-IgG1	O.talaje larvae	58
H)	O. talaje internal organs not gut	IFA 3xs	12xs-IgG1	O. talaje larvae	57
I)	None	--	--	O. turicata larvae	99
J)	O. turicata gut	IFA 3xs	--	O. turicata nymphs	99
K)	O. turicata internal organs not gut	IFA 3xs	13xs-IgG1	O. turicata nymphs	99

CFA - complete Freunds adjuvent
IFA - incomplete Freunds adjuvent
RIBA - RIBA brand adjuvent

Results

Table 7.1 lists the percent survival of ticks for each of the different immunization treatments and Figure 7.1 compares those survival percentages by treatment. Survival of the long feeding O. talaje larvae ranged from 75-78% on both naive mice and control immunized mice. Survival was reduced from that level in each of the immunization treatments C,D,E,F, G, and H and ranged from 66-53%. With the exception of treatment E, all of those treatments reduced survival of the feeding ticks by a significant amount ($p<.05$) as compared to that on the naive and control immunized mice. Treatment D consisting of three immunizations of tick gut in both complete and incomplete (2xs) Freunds adjuvent every 14 days caused the lowest survival, 53%, of engorging larvae, a 32% reduction. Survival of the fast feeding O. turicata nymphs was not affected by either of the immunization treatments J and K, remaining at nearly 100% in each case.

The antibody level of immunized mice to the respective antigen prior to feeding by the ticks is also listed in Table 7.1. At a 1:50 dilution, the titre of mice immunized with Freunds adjuvent was consistently about 15x that of control mice, showing the mice were in fact developing a strong antibody response to the respective antigens. The titre of the mice immunized with the RIBA brand adjuvent was much higher, 54xs, than that of mice immunized with Freunds, though the higher antibody titre did not cause any lower

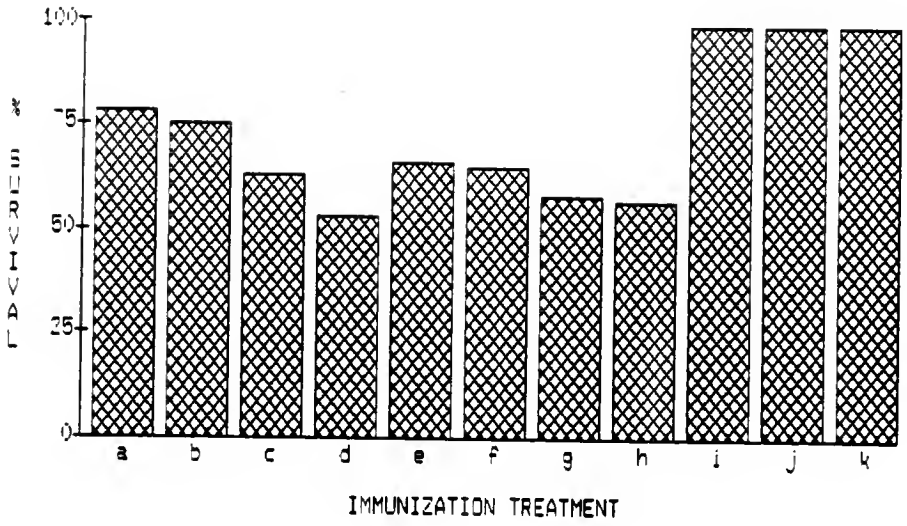

Figure 7.1. Tick survival following various host immunization procedures.

tick survival upon testing than those mice immunized with Freunds. When, in the case of treatments G,H, and K, the antibody response to immunization was isotyped, the subclass making up virtually all of the antibody response was IgG1.

When hemolymph and coxal gland secretions of O. turicata nymphs fed on mice immunized with treatment K were analyzed for the presence of isotype specific anti-O. turicata-internal-organ mouse antibody, specific mouse IgG1 was very evident in the hemolymph, though not detected in the coxal gland secretions. Though the amount of antibody present in the hemolymph was not determined, the speed with which it was detected indicates fairly high levels were present in the tick hemolymph. Specific mouse antibody was not detected in the hemolymph or coxal gland secretions of ticks feeding on a non-immunized control mouse.

Discussion

Immunization of mice with various crude extracts of O. talaje body parts did significantly ($p<.05$) lower the survival of long feeding O. talaje larvae feeding on those animals at a later date. A reduction from 78% to 53% survival was noted in this study. While this is a significant reduction in tick survival, it is the same level of reduction of survival reported in Chapter 2 resulting from three sequential exposures and the development of acquired host immunity. Therefore, the process of host

immunization used in this study to lower subsequent tick survival does not appear to be any more effective than that naturally acquired through sequential feedings. In fact, the immunization process may simply be acting to accelerate that immunity already shown to develop in response to repeated exposures. For this to be accepted, one would have to assume that there are cross-reactive proteins present in both the salivary fluids and the various body parts used in these immunization trials.

No measureable host immunization effect was noted on the fast feeding O. turicata larvae and nymphs, even though high levels of anti-tick mouse IgG1 antibody were detected in the tick hemolymph soon after feeding on immunized mice. This provides more evidence that the reduction in survival of the long feeding larvae on immunized hosts is probably more related to the host cellular response discussed in Chapter 5 resulting from naturally acquired immunity than immunity due solely to the action of ingested "activated" mouse antibody. Though this preliminary study shows little evidence of an antibody specific effect of host immunization for the control of fast feeding argasid ticks, further more detailed immunization work should be conducted using specific proteins isolated using immuno-isolation techniques. In addition, the effect of host immunization should be more closely measured by checking factors such as tick oviposition success or the effect of several feedings on immunized hosts on cohorts of ticks.

It is interesting to note that detection of an isotype specific IgG1 in the tick hemolymph shows that not only is the antibody crossing the tick gut, but that it is remaining intact, affecting neither the Fab or Fc regions of the antibody molecule. The mechanism (probably related to water removal from the bloodmeal) that permits fairly large molecules (>100 kd) to cross a generally tightly monitored barrier such as the gut should also be investigated further. That mechanism could well be the process by which large sized material such as disease organisms enter the hemocoel of ticks.

In summary, though immunization of mice with various tick parts induced high antibody levels that could be detected in the hemolymph of recently fed ticks; in the case of the rapidly feeding O. turicata it had no effect on tick survival, and in the case of the long feeding species, O. talaje, it induced a decrease in survival comparable to that of naturally acquired resistance.

CHAPTER 8
SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

This dissertation presents the results of a study that was designed to examine host immune responses of laboratory mice to sequential feedings of two species of argasid tick. The results are presented in Figure 8.1 and can be summarized as follows:

1. Acquired host resistance does develop in laboratory mice as a result of sequential exposures to larval argasid ticks. In the case of the fast feeding O. turicata, the amount of weight gained during feeding decreased significantly the second and third times larvae were exposed to the same mice. In the case of the O. talaje larvae, which take 6-8 days to feed, mortality upon second and third exposure significantly increased when compared to that seen in ticks feeding upon naive mice. In addition, while attachment time increased, detachment weight was not affected.

2. Significant antibody responses were shown to occur in mice resulting from sequential larval argasid tick exposures. Those responses remained detectable for at least 90 days after last exposure. Antibody isotyping showed that the initial response was of the IgM class with a secondary class switch to the IgG1 subclass. Using a radio-

immunoassay, cross-reactive salivary gland proteins were found to exist between both argasid and ixodid ticks.

3. Western blot methods were used to demonstrate that several different cross-reactive proteins were present in both O. turicata, O. talaje and A. maculatum tick salivary glands as well as in a Psoroptes mite, though no proteins were common to both tick families and the mite. One protein (MW 15 KD) was cross-reactive between the argasid and ixodid tick families.

4. A histological study of both the tick attachment site and the attached tick showed that a cell mediated host response occurred in laboratory mice to sequential exposures of long feeding O. talaje larvae. A definite shift from neutrophils in the first exposure to lymphocytes in the third exposure, probably T cells, was seen at the attachment site and inside the tick gut.

5. An in vitro lymphocyte proliferation test to measure mouse T cell responsiveness to sequential O. talaje tick exposures showed that a significant T cell response occurred in mice.

6. Immunization of mice with various crude extracts of tick parts induced high host antibody levels that could be detected in the hemolymph of recently fed ticks. In the case of the rapidly feeding O. turicata, however, the ingested antibodies had no effect on tick survival. In the case of the long feeding species, O. talaje, there was a

Larvae of 2 Ornithodoros species fed 3xs on individual CD1 mice.

Acquired resistance develops, manifested by the following:

1. Sig. Ab response noted, lasting at least 90 days after last exposure.
2. Primary IgM class response Secondary IgG1 subclass response.
3. O. turicata show sig. decrease in weight gained indicating host response occurring in < 60 min., probably of allergic nature.
4. Western blotting shows several cross reactive proteins present in argasid and ixodid salivary glands as well as Psoroptes mites.

1. Sig. cellular response occurs at O. talaje attachment site, shift from first to third exposure from neutrophils to lymphocytes.
2. In vitro lymphocyte proliferation test shows sig. T-cell response to feeding by O. talaje.
3. Immunization trials have no effect on fast feeding O. turicata indicating immunization elicits a cellular, delayed hypersensitivity reaction.

Figure 8.1. Summary of host immune responses of CD1 mice to sequential feedings of two species of Ornithodoros tick.

decrease in survival comparable to that observed in naturally acquired host resistance.

As outlined here, it is clear that feeding by argasid ticks stimulates a measureable immune response in laboratory mice. Evidence has been presented that all three of the host defensive systems discussed in Chapter 1, the non specific immediate response, as well as the specific antibody and cell mediated response, are stimulated. What is not clear is which of these mechanisms is responsible for the development of acquired resistance in the mice in response to the tick feedings.

This work was conducted to find out if mice respond immunologically to argasid ticks, not how they respond. The results clearly show that mice do respond immunologically. Without specific studies in which either the antibody or the cell mediated mechanisms are temporarily suppressed, it is not possible to pinpoint the exact mechanism responsible for the development of acquired resistance. Such work should be conducted. Since both of the specific mechanisms appear to be active, the possibility that antibody dependent cell mediated cytotoxicity is involved can not be ruled out. On the other hand some consideration must be given to the fact that the only mechanism that could affect the amount of weight gained by the fast feeding O. turicata larvae is that of an antibody mediated allergic nature.

While the results of the immunization trials were not as promising as hoped, these studies were very preliminary

and should receive a more concerted effort before a final decision regarding the feasibility of immunizing host animals for the control of fast feeding argasid ticks is made. Recent successes in developing immunization techniques for the control of ixodid ticks are too promising for this technique to be prematurely discarded. Having both fast and slow feeding argasid ticks provides a perfect system for delineating whether resistance due to immunization is acting by activated antibody or simply by accelerating normal acquired resistance mechanisms. Finally, the mechanism permitting host antibody to enter the tick hemolymph may be related to the entrance of disease organisms into the tick hemocoel and should be investigated.

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BIOGRAPHICAL SKETCH

James T. Need was born in Orange, Texas, on May 17, 1953. He graduated from Newark High School, Newark, Delaware, in 1971 and entered college at the University of Delaware. As an undergraduate he majored in biology, receiving a B.A. degree in 1975. He was accepted into a graduate program in the Department of Entomology and Applied Ecology, worked under Dr. Paul B. Burbutis, and received a M.S. degree in 1978. His research there centered on the use of Trichogramma wasps for the biological control of the European corn borer.

Upon graduation, he accepted an Area Extension Entomology position with the University of Missouri, where he gained a great deal of practical experience working in the southeastern region of that state. In 1980, he moved to a research technician position with Oklahoma State University, where he conducted research measuring the effects of drought stress on greenbug reproduction.

After much thought, he made a rather definitive change in career direction and on May 21, 1981, accepted a commission as a Lieutenant (Junior Grade) in the Medical Service Corps of the U.S. Navy and began work as a Medical Entomologist. His first assignment was at Navy

Environmental Preventive Medicine Unit No. 6, Pearl Harbor, Hawaii, where he was one of two medical entomologists providing preventive medicine support to Navy units throughout the Pacific. In 1984, he was assigned to Second Medical Battalion, 2nd FSSG, Camp Lejeune, North Carolina, where he served as senior preventive medicine/medical entomology officer to the Commanding General, 2nd FSSG.

In 1986, he was selected for out-service training and began work on a Ph.D. degree in the Department of Entomology and Nematology at the University of Florida under Jerry F. Butler. His research at Florida has centered around his dissertation study of the immune response of host animals to sequential feedings of argasid ticks. Upon graduation, he, his wife, Sharon Zeallor Need, and his two young boys, Logan and Kyle, will be moving to Lima, Peru, to a research position at the U.S. Navy's NAMRID facility. There he will be investigating various aspects of malaria and leishmaniasis.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Jerry F. Butler, Chairman
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. James L. Nation
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Donald W. Hall
Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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